Discordance between amyloid- PET and CSF biomarkers

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DISCORDANCE
between amyloid-β PET and CSF biomarkers:

Clinical and pathophysiological consequences

Juhan Reimand

A cooperation between:
Vrije Universiteit Amsterdam
Tallinna Tehnikaülikool

Discordance between amyloid-β PET and CSF biomarkers: Clinical and pathophysiological consequences

Juhan Reimand

The studies described in this thesis were carried out in cooperation between the Alzheimer Center Amsterdam (Department of Neurology, Vrije Universiteit Amsterdam, Amsterdam UMC) and Department of Health Technologies (Tallinn University of Technology). Research of the Alzheimer Center is part of the Neurodegeneration program of Amsterdam Neuroscience. Printing of this thesis was supported by Stichting Alzheimer en Neuropsychiatrie.

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Discordance between amyloid-β PET and CSF biomarkers: Clinical and pathophysiological consequences

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Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the joint doctoral degree at Tallinn University of Technology and at Vrije Universiteit Amsterdam has not been submitted for doctoral or equivalent academic degree.

Juhan Reimand

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Amüloid-β staatuse vastuolu PET-uuringul ning liikvorianalüüsil: Kliiniline ja patofüsioloogiline tähendus

JUHAN REIMAND

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CHAPTER I. Introduction

Dementia and Alzheimer's disease

Dementia poses an immense burden to society. According to the World Health Organization (WHO) report in 2018, there were an estimated 50 million people with dementia in the world and this number will increase to about 152 million by 2050.[1] On average, there will be a new case of dementia every 3 seconds. Dementia will cost humankind 1 trillion dollars per year and this number is estimated to double by 2030.[1] To the affected individual and their family, dementia is a devastating degrading disease.

The most common and well-known neurodegenerative cause of dementia is Alzheimer's disease (AD), which accounts for 50-80% of dementia cases.[2–4] People diagnosed with AD typically display significant memory deficits, but additional symptoms include word finding difficulties or speech comprehension disturbances, difficulties in planning and completing tasks, trouble understanding images and spatial relationships, and changes in personality and mood.

The onset of symptoms is a gradual process. Subjective cognitive decline (SCD) has been proposed to represent the first symptomatic stage of AD,[5] although not all patients with SCD have AD pathology. In SCD, patients self-report a decline in cognitive performance but their cognitive tests are in the normal range. Over time symptoms of the disease become more severe and patients progress to mild cognitive impairment (MCI), where cognitive complaints can be objectivized but they do not yet interfere significantly with everyday life. In dementia, patients lose the ability to perform everyday activities. People with end-stage disease are bed-bound and need extensive care, eventually leading to death around 10 years after dementia diagnosis. Although currently no disease modifying therapies for AD exists, a timely diagnosis is important to administer appropriate care and to be able to plan for the future.

Diagnosis of Alzheimer's disease

In 1906, Alois Alzheimer first described the histological characteristics of AD, later recognized as extracellular amyloid-β plaques and intracellular neurofibrillary tau tangles.[6] These changes formed the foundation for a neuropathological diagnosis of AD, which is still considered the gold standard for AD diagnosis.[7,8] In living patients, diagnosing AD based on histological tissue is not an option, because the procedure is associated with considerable risk of complications. Therefore, AD has historically been diagnosed based on clinical criteria.[9] However, the differential diagnosis of dementia based on only clinical symptomatology is highly complex, illustrated by the finding that across expert centres, the sensitivity of the clinical diagnosis to AD for neuropathological AD change ranged from 44 to 71%, and the specificity ranged from 44 to 71%.[10]

In the past three decades, it has been possible to support the diagnostic certainty with additional diagnostic methods such as identifying brain atrophy patterns on computed tomography (CT) and magnetic resonance imaging (MRI), or AD-specific patterns of hypometabolism on [^{18}F]fluorodeoxyglucose (FDG) positron emission tomography (PET). In addition, it is now possible to identify *in vivo* presence of amyloid-β and tau pathology by cerebrospinal fluid (CSF) analysis and by PET. These AD biomarkers have improved the diagnostic capabilities and been added to current clinical diagnostic criteria published in 2011.[11,12]

The molecular mechanisms of amyloid-β

Of these biomarkers, detection of *in vivo* amyloid-β, one of the pathological hallmarks of AD, is arguably the most influential to the diagnosis of AD. Amyloid is a term used for self-assembled, low-molecular weight peptides, usually composed of fragments of larger precursor molecules.[13] Only a proportion of known amyloidoses produce fibrillar deposits in the central nervous system and of those, the majority are caused by amyloid-β.[13]

On a molecular level, amyloid-β is a peptide composed of up to 43 amino acids. These different forms of amyloid-β are the result of the slightly imprecise sequential cleavage of the amyloid precursor protein (APP).[14] The function of APP is unclear, but it has been suggested to have a role in proliferation of fibroblasts.[15] If APP is sequentially cleaved by α-secretase and γ-secretase, short hydrophilic amyloid peptides are produced, which do not aggregate or deposit.[13] However, if sequentially cleaved by β-secretase (also called BACE1) and γ-secretase, longer peptides are produced.[14,16] Of them, the 40 and the 42 amino acid versions are the most abundant, comprising about 80-90% and 5-10% of the amyloid-β protein, respectively.[17–19] In particular, the longer peptide form $A\beta_{42}$ is most hydrophobic and more prone to aggregate into soluble oligomers and eventually into insoluble fibrillary Aβ depositions, which is the hallmark of AD pathology.[18,20] It has also been hypothesized that the oligomeric form of amyloid-β42 is the most neurotoxic form, and the fibrillary amyloid-β depositions represent the final inert stage of the disease.[21,22]

The production of different species of amyloid-β also takes place in physiologically normal cellular conditions.[18] In genetic autosomal dominant (familial) AD, certain mutations in APP or γ-secretase coding genes have been shown to cause increased production of $A\beta_{42}$.[18] However, familial cases only account for approximately 1% of AD.[23] In the large majority of sporadic AD cases, the cause of $A\beta_{42}$ accumulation is not yet clear.[20] Possible contributing factors include senescent changes, *APOE* ε4 allele, polygenetic and environmental risk factors.[24] In addition to AD, amyloid-β may also be

found in other neurodegenerative conditions, such as cerebral amyloid angiopathy (often a co-pathology to AD)[25] and Lewy body disease.[26]

Amyloid-β as the first step of Alzheimer's disease

According to currently widely accepted AD biomarker cascade theory, amyloid-β is the first pathological step toward AD (**Figure 1**).[27,28] The accumulation of amyloid-β is followed by tau-pathology, which in turn leads to the death of neurons and loss of brain structure. Finally, clinical symptoms will only begin after those pathological processes have developed. This cascade is a remarkably slow process and accumulation of amyloid-β may precede clinical symptoms by 15-20 years.[29,30] As a high proportion of cognitively normal elderly have amyloid-β pathology (for example, 33% of 80-year-olds and 44% of 90-years)[31] these people might already be at an early stage of the AD pathological trajectory. This model is supported by isolated amyloid-β pathology being much more common in large cohorts compared to the combination of amyloid-β and tau.[32] According to the current research framework, AD is defined by the existence of amyloid-β (and tau) pathology, and having normal (negative) amyloid-β biomarkers would place an individual outside of the AD continuum.[29] Therefore, *in vivo* amyloid-β diagnostics are important for the diagnosis of AD.

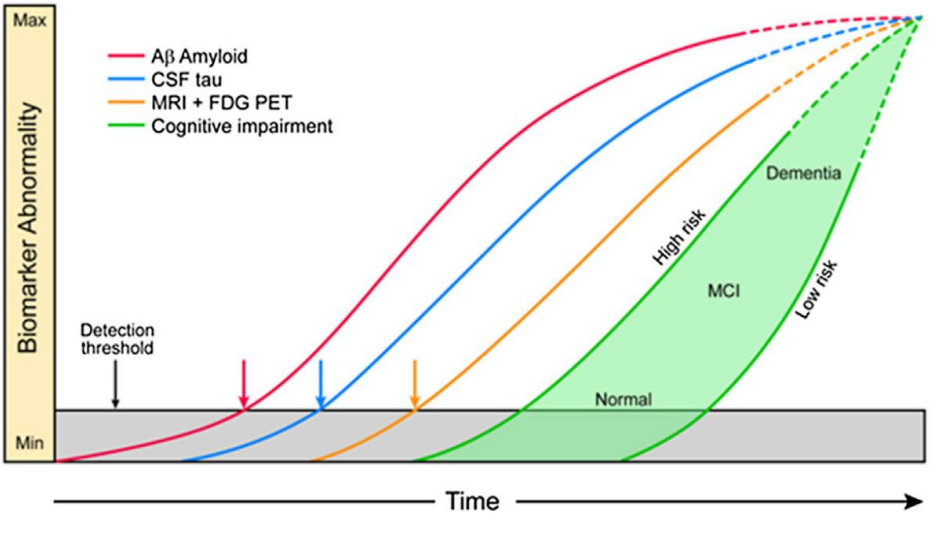

Figure 1. Dynamic biomarkers of the Alzheimer's pathological cascade[28]

Figure from Biomarker Modeling of Alzheimer's Disease; by Jack CR and Holtzman DM, Neuron, 2013, printed with permission from Elsevier.

Two methodologies for amyloid-β detection *in vivo*

It has been estimated that the total mass amyloid-β in the brain of an AD patient is about 6.5 mg, compared to 1.7 mg in subjects without AD.[33] To detect that small change *in vivo*, there are currently two established methods, i.e. by CSF analysis or by PET.

Cerebrospinal fluid

Detecting amyloid-β via CSF is based on the assessment of $A\beta_{42}$ levels in the CSF. For that, a lumbar puncture is performed to get a CSF sample, after which an immunoassay is used to detect the levels of Aβ species, most often $A\beta_{42}$. This results in a numerical value representing the total concentration of $A\beta_{42}$ in the CSF, which in turn is compared to the cohort specific cut-off value to determine amyloid-β positivity. Amyloid-β in the CSF could be first measured *in vivo* in the 1990s,[34,35] after which it was repeatedly shown that $A\beta_{42}$ levels were affected in AD.[36,37] In the 2000s, several commercial assays became available supporting the widespread use of the method.

In healthy individuals, amyloid-β proteins are detectable in the CSF, as the human organism uses CSF to clear amyloid-β from intercellular space to either lymphatics or across the blood-brain-barrier.[38] In patients with AD, however, CSF $A\beta_{42}$ levels are decreased to about half the levels seen in healthy controls.[39] Although the exact cause of the decrease of CSF $A\beta_{42}$ is not known, it is likely secondary to the increased aggregation to amyloid-β plaques, although previously changes to the production, degradation or clearance of $A\beta_{42}$ have also been hypothesized.[24,38]

Positron emission tomography

Amyloid-β can also be detected *in vivo* using PET. To that end, the patient is intravenously injected with a specific amyloid-β binding radiotracer, which circulates through the blood stream, passes the blood brain barrier and binds to fibrillar amyloid-β plaques in the brain. The tracer binding can be detected by PET scanning and be visualized and quantified after image processing. The first amyloid-β PET tracer, [^{11}C]Pittsburgh compound B (PIB), was developed in 2004 through the modification of thioflavin T, which is a fluorescent die used by neuropathologists to identify amyloid-β plaques at autopsy.[40,41] Although the usage of [^{11}C]PIB was increasingly used in academic settings, the short half-life of [^{11}C] (i.e. 20 minutes) prevented more widespread diagnostic use in clinical practice. A few years later, several new amyloid-β radiotracers emerged labelled with [^{18}F], an isotope with a longer half-life (i.e. 120 minutes) more suitable for widespread use. Most well-known [^{18}F] and FDA and EMA approved tracers include [^{18}F]florbetaben,[42] [^{18}F]flobetapir[43] and

[^{18}F]flutemetamol.[44] The high accuracy of these radiotracers in detecting *in vivo* amyloid-β pathology has been well established.[45–48]

When amyloid pathology is present, high uptake of the radiotracer is observed in grey matter relative to white matter, and absence results in a predominantly white matter uptake pattern (**Figure 2**). The clinical standard is that a trained physician (usually a nuclear medicine physician or radiologist) evaluates the scan visually and provides a dichotomous read (i.e. negative or positive for amyloid-β pathology). Alternatively, (semi)quantitative thresholds are also used in a research setting, by which the PET tracer uptake in a region of interest is quantified, intensity normalized using a reference region, and thereafter compared to a cut-off.

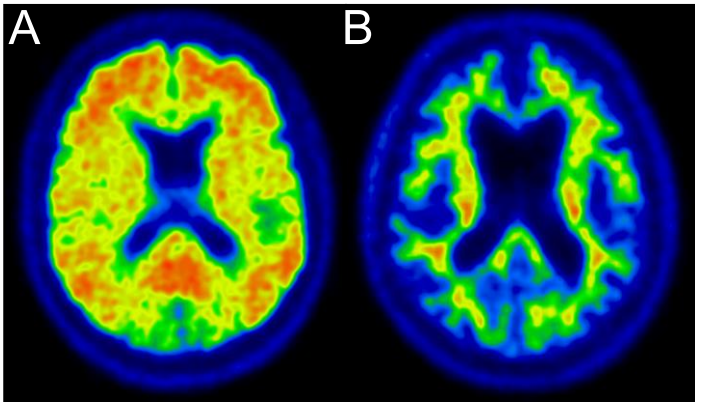

Figure 2. Examples of amyloid-β PET imaging: positive (A) and negative (B) for amyloid-β pathology

The choice between amyloid-β CSF and PET

Both CSF $A\beta_{42}$ analysis and amyloid-β PET are well-established diagnostic methods in detecting *in vivo* amyloid-β pathology, and ultimately, supporting the clinical diagnosis of AD. Therefore, these two methods are considered interchangeable in diagnostic criteria for clinical practice and research guidelines.[11,12,29] However, there are conceptual differences and both methods have their strengths and limitations.

With CSF analysis, one can measure the soluble $A\beta_{42}$, which reflects the current ratio between production and clearance of $A\beta_{42}$.[49] Therefore, CSF analysis measures the amyloid-β deposits in the brain indirectly. Compared to amyloid-β PET, CSF $A\beta_{42}$ analysis is relatively quickly accessible and cheap. The biggest benefit of CSF analysis is that with one lumbar puncture, CSF total tau (t-tau) and phosphorylated tau (p-tau) analyses are also usually performed, providing additional information about other AD hallmark pathologies. In addition, cell count, protein level and glucose can give

information about non-neurodegenerative causes of dementia. However, for CSF analysis the patient needs to undergo a lumbar puncture, which is an interventional produce, and there have been challenges with variation in methodology between centres.[50,51]

Amyloid-β PET radiotracers bind to fibrillar amyloid-β plaques in the brain, and therefore PET signal represents the total deposited amyloid-β load. With amyloid-β PET one can visualize regional tracer uptake, enabling retrieval of topographical information about amyloid-β plaques as well as visual control of the results. In addition, the digital storage of raw data allows for indefinite reanalysis and replication. However, undergoing amyloid-β PET is time-consuming, relatively expensive, and associated with ionizing radiation.[52] The accessibility of amyloid-β PET is also lower because not all centres have a PET-scanner (or access to a cyclotron).

Finally, the choice of *in vivo* amyloid-β diagnostic methods seems to also be influenced by both doctor/patient choice and cultural reasons.

Concordance and discordance between amyloid-β CSF and PET

Numerous studies have shown that the amyloid-β status determined by CSF or PET is usually concordant, i.e. the majority of cases are either amyloid-positive (CSF+/PET+) or amyloid-negative (CSF-/PET-).[52,53] Similarly, when using (semi)quantitative PET measures as a continuous variable, there is a strong inverse correlation between global cortical composite standardized uptake value ratio (SUVR) and CSF $A\beta_{42}$.[54,55] However, already in 2008 it was reported that some patients had high cortical tracer uptake without decreased CSF $A\beta_{42}$ (CSF-/PET+).[56] In a study one year later, a subset of study participants had decreased CSF $A\beta_{42}$ without increased [^{11}C]PIB cortical uptake (CSF+/PET-).[57] In the following years, the presence of this discordance between the two amyloid-β methodologies has been confirmed in multiple studies, usually amounting to about 10-20% of the cases.[58–60] Although the majority of studies primarily report the presence of the CSF+/PET- group,[57–59,61] in some publications a larger CSF-/PET+ group is also seen.[62,63]

Importance of investigating amyloid-β CSF/PET discordance

There is still much uncertainty about the cause of the amyloid-β CSF/PET discordance, as it is not known whether this is caused by biological or methodological factors, or by the combination of the two. It would be important to know whether patients with discordant amyloid-β CSF/PET status (CSF+/PET- or CSF-/PET+) have underlying amyloid-β pathology, as that would have an impact on the patient's prognosis and future care. It is also possible that the amyloid-β discordant CSF/PET status has a

separate biological basis and as such entails a different prognosis compared to people without amyloid-β or people with concordant amyloid-β detected by both modalities.

Exploring this discordance would allow us to understand whether the information provided by different amyloid-β modalities is partially different. If one amyloid-β modality were to consistently show amyloid-β pathology at an earlier stage than the other, this could be important to future trials. The majority of trials investigating possible disease modifying drugs for AD have focused on the amyloid-β pathway, and have unfortunately been unsuccessful.[64] A common theme in these trials is that they have recruited patients in late stages of the disease.[65] Previously, it has been hypothesized that amyloid-β CSF/PET discordance might be partly caused by some patients having a very early stage of amyloid-β pathology.[57,66] Therefore, if discordant amyloid-β status would be a marker for beginning AD, this could have implications in trial setups.

So far, most studies with both amyloid-β CSF/PET available have taken place in a research setting. In a clinical setting with limited resources, it is unlikely that all patients undergo two different diagnostic amyloid-β methods. However, investigating this discordance would allow to understand the clinical application of these modalities and the strengths and limitations of amyloid-β diagnostics. This is important as amyloid-β diagnostics are continuously being integrated to the clinical differential diagnosis of patients with cognitive decline. Although these two modalities are currently considered to be equal alternatives,[12,29] it is unknown, whether sometimes performing both of the modalities could give additional information.

Aims of the thesis

More specifically, the aims of this thesis were:

1. Study the clinical consequences of having discordant amyloid-β CSF/PET status.
2. Investigate whether amyloid-β CSF and PET provide partially different information.
3. Examine the clinical use of amyloid-β PET after CSF.
4. Investigate tau pathology in amyloid-β CSF/PET discordant patients.
5. Explore the neuropathological substrate for amyloid-β discordance.

Thesis outline

In this thesis we use several different approaches to explore the possible different information provided by amyloid-β PET and CSF (**Figure 3**). In **chapter II** we investigate the combined CSF/PET discordant (including both CSF+/PET- and CSF-/PET+) group and explore the clinical consequences of having amyloid-β CSF/PET discordant biomarkers in the Amsterdam Dementia cohort (ADC). Then, using the same sample, in **chapter III** we zoom in on the potential differences between CSF+/PET- and CSF-/PET+ and investigate, whether the predictive pattern of various patient features differ between the two modalities. In **chapter IV**, we explore why sometimes amyloid-β PET is clinically requested for patients with available CSF biomarker analysis in a tertiary memory clinic (Alzheimercentrum Amsterdam). Thereafter we investigate longitudinal trajectories of amyloid-β accumulation, tau and cognition and study whether amyloid-β CSF/PET discordant status is associated with tau 5 years later (**chapter V**) using Alzheimer's Disease Neuroimaging Initiative (ADNI) data. Finally, in **chapter VI**, we investigate amyloid-β CSF/PET concordance in a sample with available neuropathological data from the ADC to characterize CSF/PET discordant cases neuropathologically. We conclude this thesis by a summary of the findings, followed by methodological considerations, possible implications, and future directions (**chapter VII**).

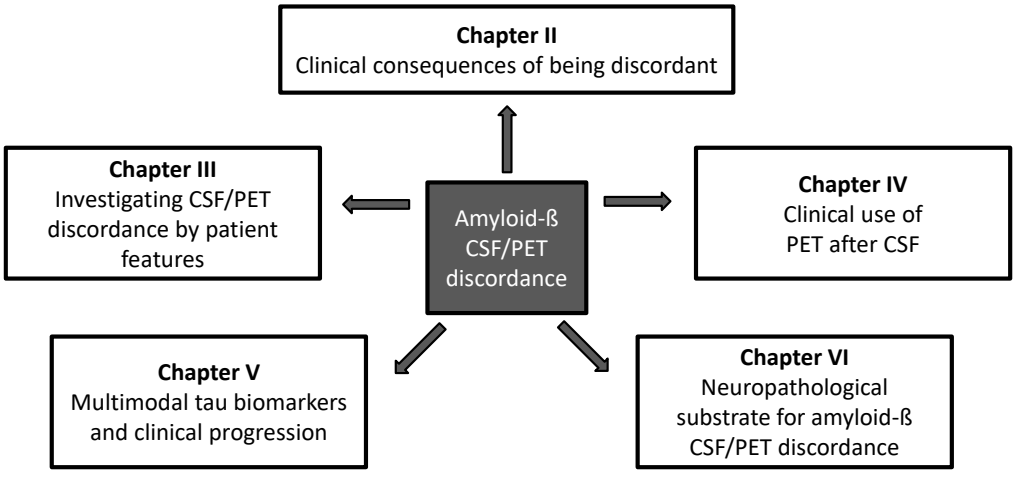

Figure 3. Thesis outline

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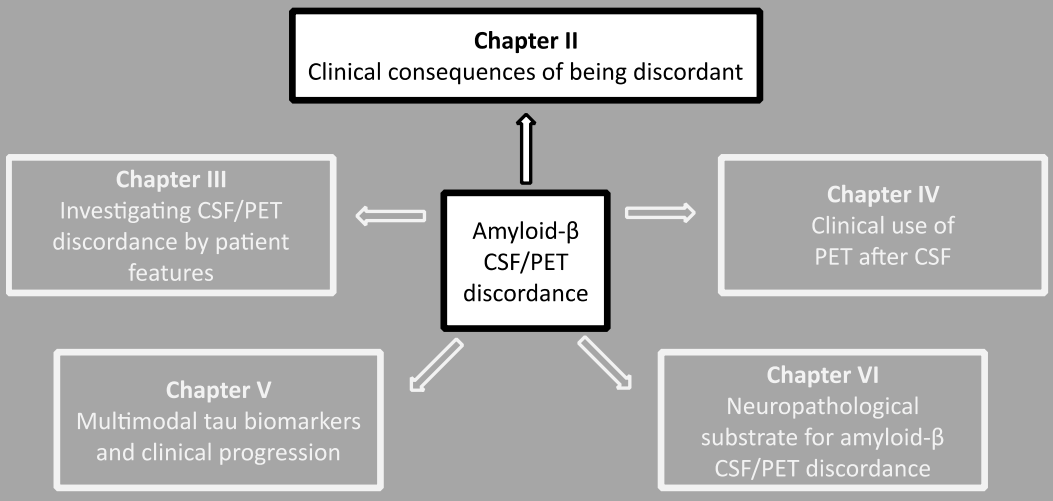
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CHAPTER II. Discordant amyloid-β PET and CSF biomarkers and its clinical consequences

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ABSTRACT

Background: In vivo, high cerebral amyloid-β load has been associated with (i) reduced concentrations of $A\beta_{42}$ in cerebrospinal fluid and (ii) increased retention using amyloid-β positron emission tomography. Although these two amyloid-β biomarkers generally show good correspondence, ~10-20% of cases have discordant results. To assess the consequences of having discordant amyloid-β PET and CSF biomarkers on clinical features, biomarkers and longitudinal cognitive trajectories.

Methods: We included 768 patients (194 with subjective cognitive decline (SCD), 127 mild cognitive impairment (MCI), 309 Alzheimer's dementia (AD) and 138 non-AD) who were categorized as concordant-negative (*n*=315, 41%), discordant (*n*=97, 13%) or concordant-positive (*n*=356, 46%) based on CSF and PET results. We compared discordant with both concordant-negative and concordant-positive groups on demographics, clinical syndrome, apolipoprotein E (*APOE*) ε4 status, CSF tau, clinical and neuropsychological progression.

Results: We found an increase from concordant-negative to discordant to concordant-positive in rates of *APOE* ε4 (28%, 55%, 70%, Z=-10.6, P<0.001), CSF total-tau (25%, 45%, 78%, Z=-13.7, P<0.001), and phosphorylated-tau (28%, 43%, 80%, Z=-13.7, P<0.001) positivity. In patients without dementia, linear mixed models showed that MMSE and memory composite scores did not differ between concordant-negative (β [SE]:-0.13[0.08], P=0.09) and discordant (β:0.08[0.15], P=0.15) patients ($P_{interaction}$=0.19), while these scores declined in concordant-positive (β:-0.75[0.08] patients ($P_{interaction}$<0.001). In patients with dementia, longitudinal cognitive scores were not affected by amyloid-β biomarker concordance or discordance. Clinical progression rates from SCD to MCI or dementia (P=0.01) and from MCI to dementia (P=0.003) increased from concordant-negative to discordant to concordant-positive.

Conclusions: Discordant cases were intermediate to concordant-negative and concordant-positive patients in terms of genetic (*APOE* ε4) and CSF (tau) markers of AD. While biomarker agreement did not impact cognition in patients with dementia, discordant biomarkers are not benign in patients without dementia given their higher risk of clinical progression.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia and is characterized by accumulation of amyloid-β (Aβ) plaques in the earliest phase of the disease.[1,2] There are currently two established methods for detecting presence of Aβ pathology *in vivo*, i.e. reduced concentrations of Aβ 1-42 ($A\beta_{42}$) in CSF and increased retention of Aβ PET tracers.[3,4] These biomarkers have been incorporated in research and diagnostic criteria.[5–8]

Within these criteria, it is assumed that CSF $A\beta_{42}$ and Aβ PET can be used interchangeably, based on mounting evidence showing strong associations between binary or continuous PET and CSF biomarkers.[9–14] Nonetheless, 10-20% of study participants have discordant results (i.e. CSF+/PET- or CSF-/PET+). Discordance in Aβ PET and CSF biomarkers potentially has important ramifications for their application in clinical, investigational or trial settings. A glimpse of this was provided by a previous study assessing longitudinal differences in cognition between participants without dementia with different CSF/PET profiles.[15] They found no memory decline in concordant-negative (CSF-/PET-) and discordant (CSF+/PET-) groups, while the concordant-positive (CSF+/PET+) group did deteriorate over time.

In the current study, we compared discordant (CSF+/PET- and CSF-/PET+) with concordant-negative (CSF-/PET-) and concordant-positive (CSF+/PET+) patients across four diagnostic groups (subjective cognitive decline (SCD), mild cognitive impairment (MCI), AD dementia and non-AD dementia) in terms of i) baseline demographics, cognition, *APOE* ε4 status and CSF tau levels, ii) longitudinal cognitive trajectories, and iii) changes in clinical diagnosis.

MATERIALS AND METHODS

Study Population

We included 768 patients who visited our tertiary memory clinic between November 2005 and November 2017 and underwent both lumbar puncture and Aβ PET within 365 days. All patients underwent a standard diagnostic evaluation consisting of medical history, informant-based history, neurological examinations, neuropsychological testing, basic laboratory testing, apolipoprotein E (*APOE*) genotyping, MRI and CSF.[16] Clinical diagnoses at baseline were established by consensus at multidisciplinary meetings using conventional diagnostic criteria, without knowledge of CSF results. Aβ PET was not part of standard diagnostic evaluation and was performed separately within the context of clinical research studies. Clinical follow-up including neuropsychological examination was performed annually. CSF and Aβ PET results

were available to clinicians at time of follow-up visits. Patients were divided into four diagnostic groups: SCD, MCI, AD and non-AD. SCD refers to patients presenting with cognitive complaints in the absence of objective cognitive decline or neurologic impairment (i.e. criteria for MCI, dementia or any neurologic or psychiatric disorder not met). Patients with a syndrome diagnosis of dementia and a suspected non-AD etiology were categorized as non-AD dementia (e.g. frontotemporal dementia, vascular dementia, dementia with Lewy bodies or progressive supranuclear palsy). Patients with a postponed or other neurological diagnosis (69 (9%) at baseline and 48 (6%) after their last visit) were included in one of the four diagnostic groups based on the probable syndrome diagnosis and suspected etiology, as indicated by the neurologist in the medical records. The closest visit with a full neuropsychological assessment within a year of the first Aβ biomarker test was considered the baseline visit.

Neuropsychological assessment

Cognitive functioning was assessed using a standardized neuropsychological test battery covering global cognition and five cognitive domains (i.e. memory, language, attention, executive and visuospatial functions).[17] For global cognition, we used the Mini-Mental State Examination (MMSE). We used the Visual Association Test (VAT) and total immediate recall and delayed recall of the Dutch Version of the Rey Auditory Verbal Learning Test for memory. For language, we used the VAT naming and category fluency (animals). For attention, we used the Trail Making Test (TMT) part A, the forward condition of the Digit Span, and the Stroop Test card I (word) and II (color). We used the TMT part B, the backward condition of the Digit Span, Stroop Test card III (word-color), Frontal Assessment Battery, and the Dutch version of the Controlled Oral Word Association Test (letter fluency) for executive functioning. Finally, we assessed visuospatial functioning using three subsets of the Visual Object and Space Perception (VSOP) battery: (i) incomplete letters, (ii) dot counting, and (iii) number location.

Neuropsychological data were transformed to z-scores, using the mean and standard deviations of 360 cognitively normal individuals (mean age ± SD: 58 ± 8, female sex: 140 (39%)), who were cerebrospinal fluid biomarker negative and visited our memory clinic between 2001 and 2015.[18] TMT A, TMT B and the Stroop Tests were log transformed to account for their non-normal distribution, and inverted by computing -1 * z-score, so that lower scores indicate worse test performance. When TMT B was aborted during the task (328/1986 (17%) observations), we estimated the TMT B by multiplying the time needed to complete the TMT A with the mean TMT B/A ratio from the respective diagnostic group. For the five cognitive domains, we calculated mean z-scores by averaging all completed tests in each domain. A domain z-score was generated if a patient had completed a minimum of one test per domain. The proportions of missing neuropsychological test results are shown in **Supplementary**

Table 1. At least one follow-up visit was available for 538 (70.0%) patients. The median follow-up time was 1.9 (IQR 1.1 – 2.7) years.

CSF

We obtained CSF by a lumbar puncture between L3/4, L4/5 or L5/S1 intervertebral space, using a 25-gauge needle and a syringe.[16] We collected the samples in polypropylene microtubes, centrifuged at 1800g for 10min at 4°C. Thereafter the samples were frozen at -20 °C until manual analysis of $A\beta_{42}$, total tau and tau phosphorylated at threonine 181 (p-tau) using sandwich ELISAs [Innotest assays: β-amyloid 1-42, tTAU-Ag and PhosphoTAU-181p; Fujirebio (formerly Innogenetics)] at the Neurochemistry laboratory of the Department of Clinical Chemistry of VUmc. As the median CSF $A\beta_{42}$ values of our cohort have been gradually increasing over the years, we corrected all $A\beta_{42}$ values to adjust for the longitudinal upward drift.[16] In short, based on the cross-section of bimodal distributions of $A\beta_{42}$ concentrations in our memory clinic cohort, year-specific cut points were determined with Gaussian mixture modeling. By this approach, every $A\beta_{42}$ value in the total Amsterdam Dementia Cohort was retrospectively modified to adjust for the drift, allowing to use a uniform $A\beta_{42}$ cut-off value of < 813 pg/mL. This method was validated using three different approaches, of which one was by calculating its concordance with amyloid PET results (88%). Cut-off values for total tau and p-tau were > 375 pg/mL, and > 52 pg/mL respectively.[19]

PET

Aβ PET is not routine in our diagnostic work-up but is usually performed as part of research programs or sometimes as an add-on diagnostic test.[16] We performed Aβ PET on either the Gemini TF PET-CT, Ingenuity TF PET-CT, Ingenuity PET/MRI system (all Philips Medical Systems. Best, The Netherlands), and ECAT EXACT HR+ scanner (Siemens Healthcare, Erlangen. Germany) PET scanners. We included 271 (35%) patients who underwent PET using [^{11}C]PIB, 24 (3%) using [^{18}F]florbetapir, 151 (20%) using [^{18}F]flutemetamol, and 322 (42%) using [^{18}F]florbetaben. All acquisition and processing procedures have been described in detail elsewhere.[20–25] For all PET scans, whole-brain visual assessment was performed by an experienced nuclear medicine physician (BvB), according to guidelines approved by the FDA ([^{18}F]florbetapir, [^{18}F]flutemetamol, and [^{18}F]florbetaben) or as described previously ([^{11}C]PIB).[21,22,25] Scans were rated as positive or negative for the presence of Aβ pathology. Aβ PET scans were performed within a median of 54 (IQR 14 - 75) days of the lumbar puncture.

Classification of patients

Based on CSF $A\beta_{42}$ and Aβ PET results, patients were categorized into three groups: concordant-negative (PET-/CSF-), discordant (combined CSF+/PET- or CSF-/PET+) or concordant-positive (CSF+/PET+).

Statistical analysis

Statistical analysis was performed using R software (Version 3.4.3, The R Foundation for Statistical Computing). We compared baseline demographic, clinical and cognitive characteristics between discordant and concordant (both negative and positive) patients within each diagnostic group, and used Chi-squared tests, two samples *t*-tests and Wilcoxon Rank-Sum tests where appropriate. We calculated the overall concordance rate between Aβ PET and CSF $A\beta_{42}$ as a percentage of concordant patients of the whole study population. To validate the concordance rate, we performed receiver operating characteristic (ROC) analysis to calculate the area under the curve (AUC) of the CSF total tau/$A\beta_{42}$ ratio for amyloid PET positivity. Note that we used the drift-adjusted CSF $A\beta_{42}$ values, but the original CSF tau values, as the drift in time only pertained to measurements of $A\beta_{42}$.[26] We defined the cut-point (0.44) that maximized the Youden index for amyloid PET positivity and calculated diagnostic accuracy.[27] We used Chi-squared tests to assess differences in proportions of discordance between the different Aβ PET tracers. To examine trends for increased proportions of *APOE* ε4 carriership, levels of CSF total tau and p-tau, and diagnostic conversion (both progression and regression) from concordant-negative to discordant to concordant-positive, we used the Cochrane-Armitage trend test.[28] For these analyses, we dichotomized levels of CSF total tau and p-tau for consistency (see “CSF” section).

We used linear mixed models to assess changes in domain-specific neuropsychological z-scores and MMSE scores over time, stratifying for patients with and without dementia, comparing discordant patients with both concordant-negative and concordant-positive groups. We used a random intercept with a fixed slope, and adjusted for age, sex and education. The models further included terms for time and CSF/PET profiles, as well as an interaction term time x CSF/PET profiles. Data are presented as β coefficients (SE), reflecting annual change in composite z-scores. The P value for slope represents the significance of the interaction between time and group, separately analyzed within groups (concordant-negative, discordant and concordant-positive). The P value for interaction represents the significance of the interaction between time and concordant-negative and concordant-positive groups with the discordant group as reference. We performed Bonferroni correction for group-wise testing on all comparisons between concordant and discordant groups and applied a significance level of $P < 0.05$.

Standard protocol approvals, registrations, and patient consent

The institutional review board of the VU University Medical Center approved all individual studies from which the current data was gathered and retrospectively analyzed. All patients provided written informed consent for their data to be used for research purposes.[20–25]

Data availability statement

All published and unpublished anonymized data from this study can be made available upon reasonable request from a qualified investigator to the corresponding author.

RESULTS

Discordance between Aβ CSF and PET

Across all groups, discordance between CSF and PET was n = 97 (13%). When discordant, CSF was more often positive then PET (67% vs. 33%, P < 0.001). The proportion of patients with a discordant CSF/PET profile varied between diagnostic groups, but was not significantly different (SCD 16%, MCI 13%, AD dementia 9%, and non-AD dementia 16%, P = 0.13) (**Table 1**). When excluding patients with a CSF value within 5% (range: 773-853 pg/mL) or 10% of the cut-off value (range: 732-894 pg/mL), the overall discordance decreased from 13% to 11% to 9% respectively. This indicates that accounting for threshold issues lowers biomarker discrepancies, but concordance remained at a similar level. The decrease in discordance was most prominent in patients with AD dementia (from 9 to 5 to 5%, **Table 1** and **Figure 1**). We also examined PET-CSF discordance using the CSF total tau/$A\beta_{42}$ ratio, using a cut-off derived from predicting amyloid PET positivity. Similarly, this resulted in overall 13% PET-CSF discordance (14% in SCD, 9% in MCI, 9% in AD dementia and 24% in non-AD dementia).

The proportion of patients with a discordant CSF/PET profile across the different Aβ PET tracers varied between 9% and 17% but was not significantly different (P = 0.53) (**Supplementary Figure 1**). A total of 47/768 (6%) had repeated (≥2) amyloid PET scans, of which 28 patients were scanned using different tracers. Amyloid PET result changed in only 3 patients over time, going from negative to a positive result.

Table 1. Rate of discordance across diagnostic groups

	Total	SCD	MCI	AD dem	Non-AD dem
N (%)	768	194 (25)	127 (17)	309 (40)	138 (18)
Discordant, cut-off < 813 ng/L (%)*	97 (13)	30 (15)	17 (13)	28 (9)	22 (16)
CSF+/PET- (%)	65 (67)	20 (67)	9 (53)	17 (61)	19 (86)
Discordant, excl. ±5% cut-off (%)	75 (11)	27 (15)	14 (12)	15 (5)	19 (15)
Discordant, excl. ±10% cut-off (%)	56 (9)	20 (12)	10 (10)	13 (5)	13 (11)

Abbreviations: AD, Alzheimer's disease; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; PET, positron emission tomography; SCD, subjective cognitive decline. *Proportion of discordant patients between diagnostic groups does not differ significantly (Chi squared test).

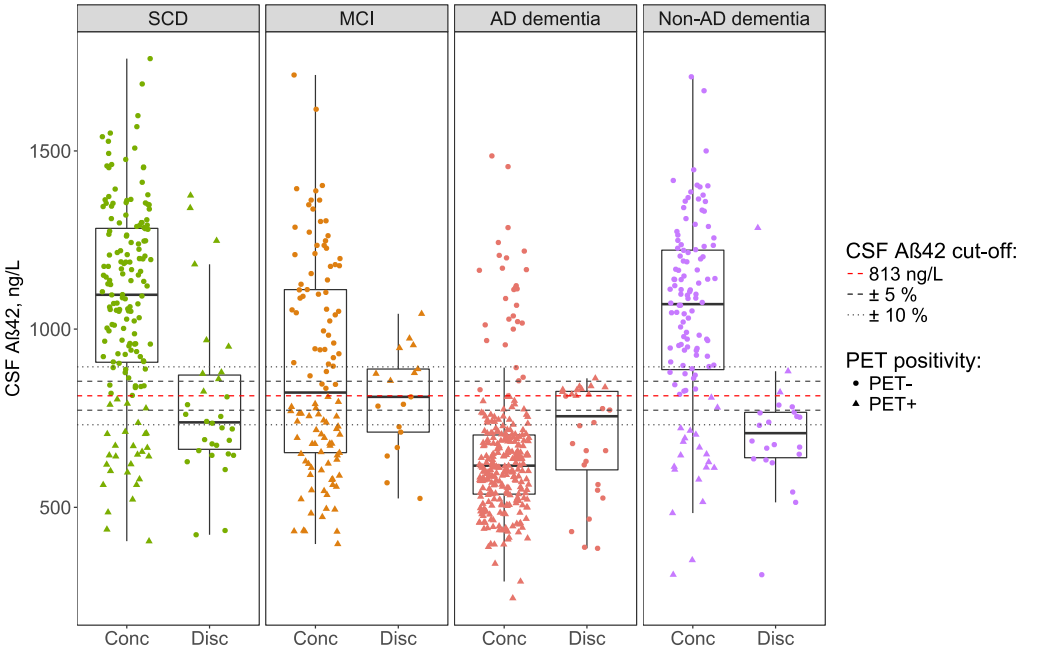

Figure 1. Distribution of CSF $A\beta_{42}$ CSF/PET discordant and concordant patients per syndrome diagnosis

Abbreviations: AD, Alzheimer's disease; CSF, cerebrospinal fluid; Conc, concordant; Disc, discordant; MCI, mild cognitive impairment; SCD, subjective cognitive decline.

Baseline characteristics

Of all patients (n=768), 194 (29%) had SCD, 127 (17%) MCI, 309 (40%) AD dementia, and 138 (18%) non-AD types of dementia (**Table 2**). The non-AD type dementia group included the frontotemporal dementia spectrum (66, 48%), dementia with Lewy bodies (22, 16%), vascular dementia (6, 4%) and other dementia syndromes (44, 32%) like progressive supranuclear palsy and corticobasal syndrome. Overall, discordant patients did not differ in age, sex and education from concordant-negative and concordant-positive patients. At baseline, discordant patients had lower scores for MMSE, and the cognitive domains memory, language, and visuospatial than concordant-negative patients. In contrast, discordant patients performed better on MMSE and the memory domain than concordant-positive patients.

APOE ε4, CSF total tau and p-tau levels

Figure 2a shows the distribution of *APOE* ε4 status in discordant and concordant patients across the whole sample, as well as its distribution within the different diagnostic groups. Trend analyses showed that there is an increase of the proportion of *APOE* ε4 positivity from concordant-negative to discordant to concordant-positive, across the whole sample (Cochrane-Armitage trend test Z-score = -10.6). *APOE* ε4 positivity was comparable between CSF+/PET- and CSF-/PET+ groups (52% versus 60%. P = 0.65). Similarly, APOE ε4 positivity was not a significant predictor (P = 0.49) in a logistic regression model involving only the CSF/PET discordant population (n = 97) with discordant group status (either CSF+/PET- or CSF-/PET+) as the outcome. There was a similar trend within SCD (Z = -3.9), MCI (Z = -6.4) and AD dementia (Z = -3.8) (all P < 0.001), but not in the non-AD group (Z = -1.3, P = 0.18). Analyses for dichotomized CSF total tau (cut-off: >375 pg/mL) (**Figure 2b**) and CSF p-tau (cut-off: >52 pg/mL) (**Figure 2c**) showed the same trend across the whole sample (total tau: Z = -13.7, p-tau: Z = -13.6) and within SCD (total tau: Z = -5.5, p-tau: Z = -3.9), MCI (total tau: Z = -5.0, p-tau: Z = -5.6) and AD dementia (total tau: Z = -5.6, p-tau: Z = -6.1) (all P < 0.001), as discordant patients had higher CSF total tau and p-tau levels than concordant-negative patients, while concordant-positive patients had higher CSF total tau and p-tau levels than discordant patients.

Longitudinal cognitive trajectories

Next, we performed linear mixed models to examine cognitive changes over time. Results are presented for the non-dementia (SCD and MCI combined) and dementia (combined AD and non-AD dementia) groups (**Figure 3** and **Supplementary Table 2**).

Table 2. Baseline demographic and clinical characteristics

	Total (N = 768)			SCD (N = 194)			MCI (N = 127)			AD dementia (N = 309)			Non-AD dementia (N = 138)		
CSF / PET profile	- / -	Disc.	+ / +	- / -	Disc.	+ / +	- / -	Disc.	+ / +	- / -	Disc.	+ / +	- / -	Disc.	+ / +
N (%)	315 (41)	97 (13)	356 (46)	136 (70)	30 (15)	28 (14)	55 (43)	17 (13)	55 (43)	28 (9)	28 (9)	253 (82)	96 (70)	22 (16)	20 (14)
Age (SD)	63 (8)	63 (9)	64 (7)	60 (7)	60 (7)	61 (9)	67 (7)	66 (9)	64 (8)	65 (7)	65 (8)	63 (7)	64 (8)	63 (9)	67 (5)
Sex, male (%)	211 (67)	58 (60)	192 (54)	85 (63)	20 (67)	11 (39)	43 (78)	10 (59)	32 (58)	20 (71)	13 (46)	136 (54)	63 (66)	15 (68)	13 (65)
Education (IQR)	5 (4-6)	5 (4-6)	5 (4-6)	6 (5-6)	5 (4-6)	6 (5-7)	6 (5-6)	6 (5-6)	5 (5-6)	5 (4-6)	5 (4-6)	5 (4-6)	5 (4-5)	5 (4-5)	6 (5-6)
MMSE (SD)	26 (3)[c]	24 (4)	23 (4)[b]	28 (2)	27 (3)	28 (3)	27 (2)	26 (3)	27 (2)	24 (3)	22 (4)	22 (4)	24 (4)	23 (5)	24 (4)
<u>Cognitive domains (Z-scores):</u>															
Memory (SD)	-1.4 (2.3)[c]	-2.5 (2.9)	-3.3 (2.8)[a]	-0.3 (0.9)[a]	-0.9 (1.7)	-0.3 (1.0)	-1.6 (2.0)	-2.1 (1.8)	-2.3 (1.8)	-3.4 (2.3)	-4.0 (3.5)	-4.0 (2.8)	-2.3 (2.9)	-3.0 (3.1)	-2.3 (2.1)
Language (SD)	-0.7 (1.3)[b]	-1.3 (2.1)	-1.0 (1.8)	-0.1 (0.8)	-0.2 (0.5)	0.0 (0.5)	-0.5 (0.7)	-0.8 (0.8)	-0.2 (0.4)[b]	-1.3 (1.3)	-1.9 (2.2)	-1.3 (1.9)	-1.4 (1.7)	-2.3 (3.0)	-2.0 (2.9)
Attention (SD)	-0.7 (1.1)	-0.9 (1.0)	-1.1 (1.2)	-0.2 (0.8)	-0.5 (1.0)	-0.2 (1.3)	-0.5 (0.8)	-0.6 (1.0)	-0.3 (0.7)	-1.2 (1.1)	-1.3 (0.9)	-1.4 (1.2)	-1.4 (1.2)	-1.4 (1.0)	-1.4 (1.0)
Executive (SD)	-1.0 (1.4)	-1.3 (1.4)	-1.5 (1.4)	-0.2 (1.0)	-0.5 (1.3)	-0.1 (1.0)	-0.8 (0.9)	-0.6 (0.9)	-0.5 (0.9)	-1.9 (1.1)	-2.1 (1.1)	-1.9 (1.3)	-2.1 (1.3)	-1.9 (1.4)	-1.9 (1.3)
Visuospatial (SD)	-0.3 (1.2)[a]	-0.9 (1.8)	-1.4 (2.4)	0.0 (0.6)	-0.4 (1.8)	0.0 (1.0)	-0.3 (1.0)	-0.7 (1.1)	-0.1 (1.0)	-0.8 (1.3)	-1.4 (2.1)	-1.8 (2.6)	-0.8 (1.6)	-1.2 (1.5)	-1.2 (1.4)

Abbreviations: AD, Alzheimer's disease; CSF, cerebrospinal fluid; IQR, interquartile range; MCI, mild cognitive impairment; PET, positron emission tomography; SCD, subjective cognitive decline; SD, standard deviation. Data are presented as No. (%), mean (SD) or median (IQR). Within diagnostic groups, we calculated differences between discordant and both concordant groups. Education was unavailable for 28 (4%) patients, APOE genotype for 32 (4%), and MMSE for 15 (2%). Based on missing data, we could not construct a Z-score for *n* (%) patients for the following domains: 41 (5%) for memory, 48 (6%) for language, 43 (6%) for attention, 21 (3%) for executive functioning, and 67 (9%) for visuospatial functioning. [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$

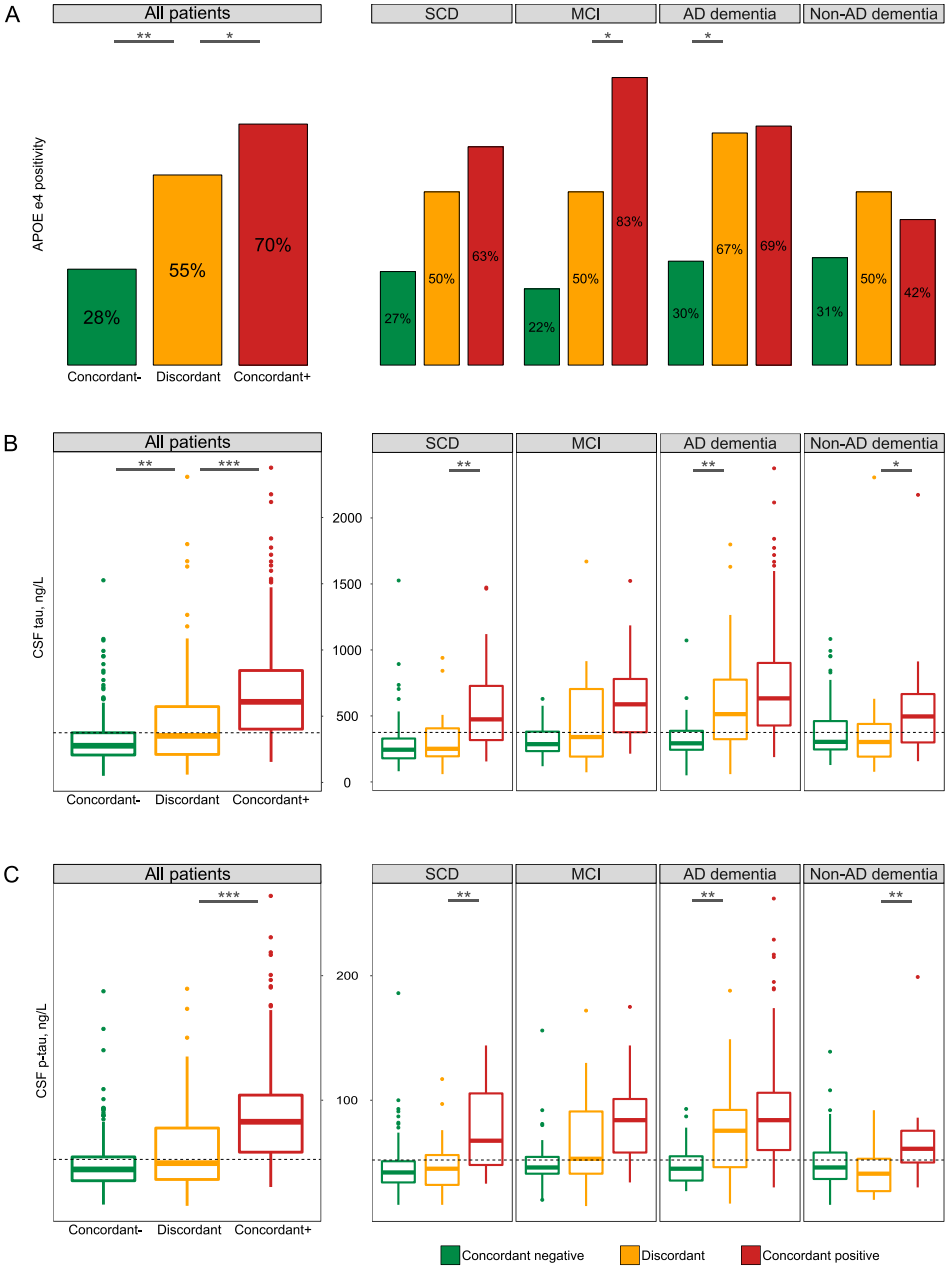

Figure 2. Differences in *APOE* ε4 genotype, CSF total tau and phosphorylated tau levels between discordant and concordant patients

Abbreviations: AD, Alzheimer's disease; APOE, apolipoprotein E; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; SCD, subjective cognitive decline. Dotted lines on boxplot graphs represent clinical cut-offs for CSF total tau (375 ng/L) and phosphorylated tau (52 ng/L). Significance levels for group comparisons: * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$.

In the non-dementia group, there was no difference in MMSE score over time between discordant (β: 0.08 [0.15]; P for slope = 0.56) and concordant-negative patients (β: -0.13, [0.08]; P for slope = 0.09; P for interaction = 0.19), while discordant patients performed better than concordant-positive patients (β [SE]: -0.75, [0.08]; P for slope <0.001; P for interaction <0.001). Results for longitudinal decline in memory function were similar, as discordant (β: -0.03 [0.09]; P for slope = 0.78) and concordant-negative patients (β: -0.04 [0.05]; P for slope = 0.38, *P* for interaction = 0.87) did not differ, while discordant patients demonstrated less decline than concordant-positive patients (β: -0.53 [0.05]; P for slope < 0.001; *P* for interaction = <0.001). In addition, discordant patients β: 0.02 [0.04]; P for slope = 0.68) had better attention scores over time than concordant-positive patients (β: -0.10 [0.03]; P for slope < 0.001; P for interaction = 0.02). There were no group differences in the remaining domains (i.e. language, executive, and visuospatial). In patients with dementia, the rates of cognitive decline as measured by MMSE and composite z-scores of the five cognitive domains did not differ between concordant or discordant groups.

Impact of biomarker concordance on changes in clinical diagnosis during follow-up

The frequency of change in syndrome diagnosis, from SCD to MCI or dementia (Z = -2.6, P = 0.01), and from MCI to dementia (Z = -3.0, P < 0.01), increased with the addition of a positive Aβ marker (i.e. from concordant-negative to discordant to concordant-positive, **Figure 4a**). Conversely, regression from dementia to MCI or SCD increased with the absence of a positive Aβ marker (Z = 5.1, P < 0.001), while we observed a similar trend in MCI for regression to SCD (Z = 2.2, P = 0.03 (**Figure 4b**).

Figure 5 shows changes in clinical diagnosis, which occurred in 134 (17%) patients during a median follow-up time of 1.9 (IQR 1.1 – 2.7) years. These changes were similar in discordant (*n*=22, 23%) and concordant-negative (*n*=65, 21%) patients, but occurred less frequent in concordant-positive patients (n=47, 13%) compared to discordant patients at statistical trend level (P = 0.062). In discordant patients, only 5 (23%) changes were towards a diagnosis of probable AD, while the majority of changes (*n*=36, 77%) were towards AD in concordant-positive patients. In concordant-negative patients, there was no clear pattern in the changes of clinical diagnosis. The increasing spread in distribution of diagnostic changes in patients with discordant and concordant-negative profiles suggests that the absence of a clear Aβ positive profile makes clinical decision-making less straightforward.

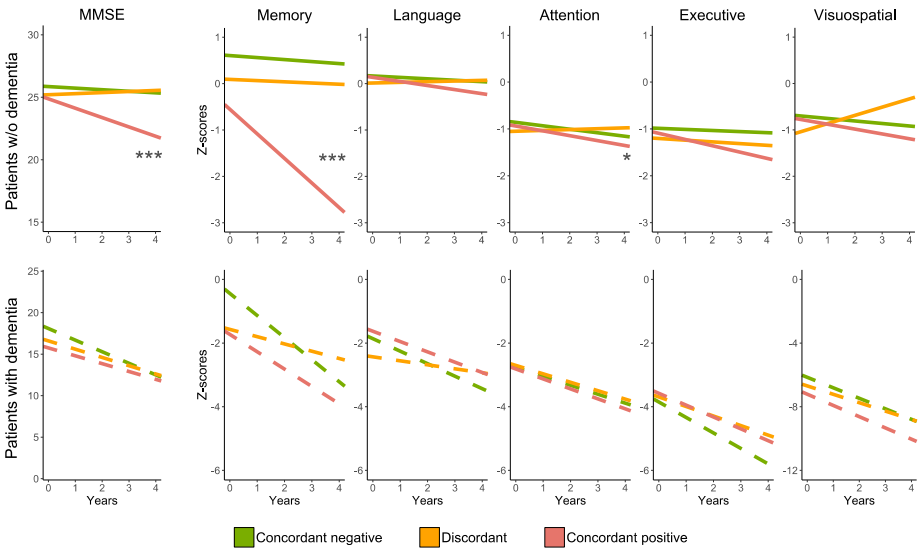

Figure 3. Cognitive trajectories of patients without and with dementia based on discordance and concordance.

0-5% of data points for MMSE and 0-2% of data points for z scores (memory, language, attention, executive, visuospatial) lie outside of the time range visualized on graphs. Significance levels for group comparisons: *** p < 0.001.

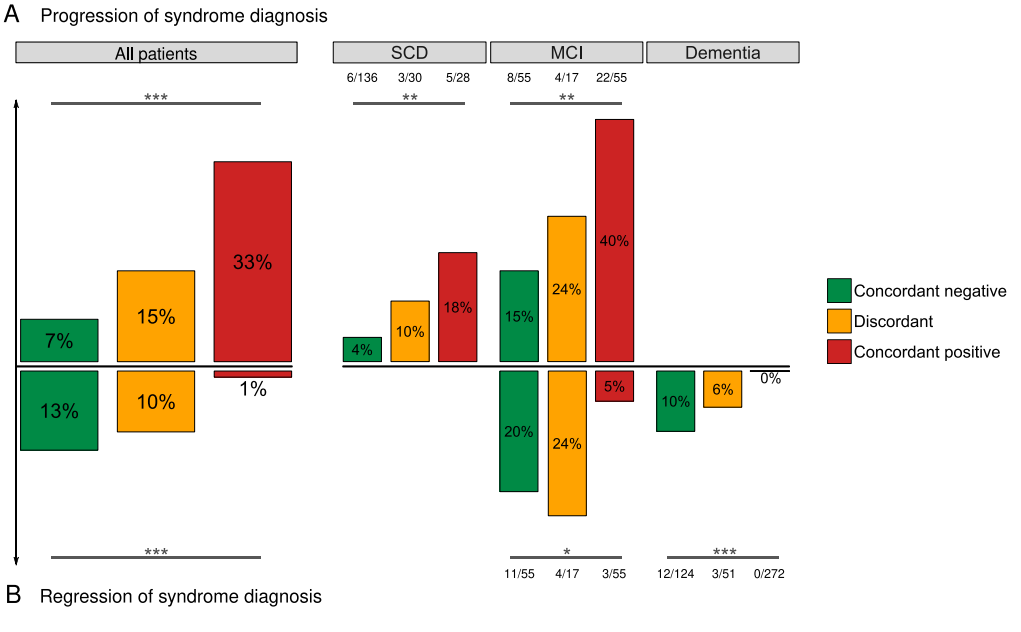

Figure 4. Differences in change of syndrome diagnosis between discordant and concordant patients

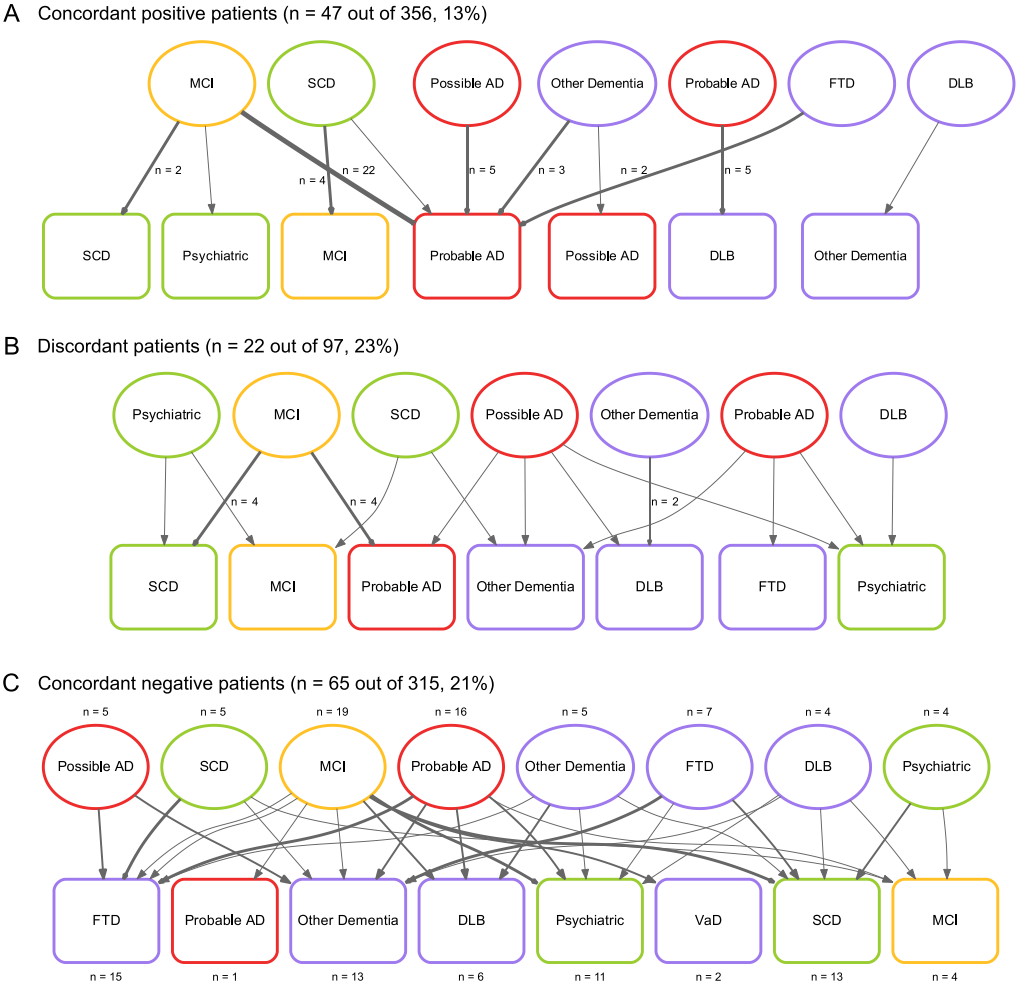

Figure 5. Changes of clinical diagnosis during follow-up based on discordance and concordance

Abbreviations: AD, Alzheimer's disease; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; MCI, mild cognitive impairment; SCD, subjective cognitive decline; VaD, vascular dementia.

DISCUSSION

In the present study, we found that patients with discordant Aβ PET and CSF markers were intermediate to concordant-negative and concordant-positive groups on genetic (*APOE* ε4 positivity) and CSF (tau) markers of AD. In patients without dementia (SCD and MCI combined), discordant cases performed similar to concordant-negative cases in memory function and global cognition, while concordant-positive cases showed a steeper decline. Furthermore, there was an increase in the proportion of patients

demonstrating change in syndrome diagnosis (from SCD to MCI or dementia, or from MCI to dementia) from concordant-negative to discordant to concordant-positive groups. In patients with dementia (AD and non-AD syndromes combined), Aβ biomarker discordance or concordance did not affect cognitive trajectories. Altogether, our findings suggest that discordant Aβ biomarkers provide important diagnostic and prognostic information in individuals without dementia.

Aβ pathology can be reliably measured *in vivo* using PET or in CSF, but there exists substantial discordance between these markers when obtained in the same individuals (~10-20% in the literature, 13% in the current study). However, whether and how Aβ biomarker discordance affects clinical progression or diagnostic changes is currently understudied. We showed that discordant patients without dementia (SCD and MCI combined) had favorable trajectories on memory and global cognitive functions compared to concordant-positive cases, which is in line with earlier studies.[15] However, compared to concordant-negative cases, patients with discordant Aβ markers were at increased risk of diagnostic progression (from SCD to MCI or dementia, or from MCI to dementia). This indicates that although the prognosis is better than in patients with two abnormal Aβ markers, positivity on a single marker in patients without dementia is not benign.

At the dementia stage, Aβ biomarker agreement did not have an effect on cognitive changes over time, as there were no differences in slopes between the concordant and discordant groups. This suggests that the relative contribution of amyloid-β pathology to cognitive impairment is limited at more advanced disease stages,[29–31] and is presumably driven by other processes including accumulation of tau pathology and cerebrovascular disease. Despite the absence of an effect on cognition, biomarker discordance does seem to affect clinical decision-making, as the proportion of changed diagnoses was higher in discordant (and concordant-negative) cases compared to concordant-positive patients. This is likely due to the awareness of clinicians that a negative Aβ biomarker (even when the other marker is positive) makes the diagnosis of AD less probable.[32] This would often require a diagnostic change when AD was the initial clinical diagnosis. In contrast, positive Aβ biomarkers in non-AD syndromes do not necessarily mandate a diagnostic change, because Aβ could be considered comorbid to a primary pathology that drives the clinical presentation.[33,34] This study suggests simultaneous assessment of Aβ PET and CSF biomarkers provide complementary information to clinicians in certain diagnostic (i.e. differential diagnosis in patients with dementia) and prognostic (i.e. predicting clinical progression in patients without dementia) scenarios.

Among groups, discordant cases had higher rates of CSF tau and *APOE* ε4 positivity compared to concordant-negative cases. In the SCD group, this might indicate that discordant cases are further along the disease pathway and more “AD-like” than

concordant-negative cases. At this early stage when Aβ burden is still relatively low, presence of Aβ might be detected earlier by one of the modalities, leading to a discordant profile. At the MCI and especially dementia stage when clinical symptoms are expressed, however, they should have significant Aβ burden that would be detected by both modalities. Yet, there was substantial discordance, especially in non-AD types of dementia. This might be explained by i) presence of Aβ at relatively low levels as a comorbid pathology in the non-AD group, ii) some individuals may have low resilience against Aβ pathology and show cognitive deficits at low levels of Aβ,[35] iii) differences in Aβ morphology that hampers detection by one of the modalities,[36,37] or iv) several methodological aspects that are discussed in the paragraph below.[5–8,38] Discordant Aβ markers have frequently been explained by suboptimal thresholds for Aβ-positivity. For example, increasing the cut-off value for CSF $A\beta_{42}$ positivity in CSF (possibly at the expense of reduced sensitivity) can increase concordance rates between PET and CSF by tipping over cases with borderline positive results.[39] Furthermore, CSF $A\beta_{42}$ to $A\beta_{40}$ ratios can also improve concordance rates between CSF and PET, as this accounts for interindividual variability in Aβ production, CSF turnover or pre-analytical influences such as absorption.[10,40,41] The immunoassays that are being used might also explain some variance of discordance, as newer immunoassays show improved agreement between CSF and PET.[42] On the PET side, visual read metrics and quantitative threshold approaches to determine Aβ PET positivity are affected by several factors (e.g. partial volume effects, non-specific binding or reconstruction artefacts) that could lower their accuracy.[10,43] Nevertheless, when we excluded cases within 5% or 10% around the CSF $A\beta_{42}$ cut-off value of 813 ng/L relatively high discordance rates (11% and 9%) were still observed, suggesting that only a small proportion of discordant cases are explained by threshold definitions [44].[44] Several alternative mechanisms have been proposed that could help explaining discordance between Aβ PET and CSF biomarkers. First, the majority of discordant cases are CSF+/PET-, with the highest proportion of CSF+/PET- profiles observed in cognitively normal individuals.[10–13,45–47] Consequently, it was hypothesized that Aβ accumulation may be detected earlier in CSF than by Aβ PET in preclinical AD.[12,14,15,46,48] We found a similar, non-significant, trend with the highest proportion of discordant cases in the SCD and non-AD dementia groups, who are presumably at earlier phases of (age-related or comorbid) Aβ accumulation compared to MCI and AD dementia patients. Second, isolated Aβ-positivity in CSF could be caused by other conditions unrelated to AD pathophysiology, such as cerebrovascular disease, neuroinflammation or amyotrophic lateral sclerosis.[15,49,50] A third explanation is the presence of analytical artefacts, as CSF $A\beta_{42}$ might adsorb onto tube surfaces, which decreases available $A\beta_{42}$ for analysis,[51] while PET may yield false positive results in patients with cerebral amyloid angiopathy and false negative results in patients with atypical forms of Aβ pathology.

Strengths of this monocenter study include the large sample size with both Aβ PET and CSF data in a clinically relevant memory clinic population and the availability of longitudinal cognitive and clinical data. There are also several limitations. First and foremost, the retrospective study design (data were collected between November 2005 and November 2017) could have led to several sources of bias that we could not account for. Second, despite the large sample size the discordant group was relatively small (n=97), especially when considering that these patients were distributed across four different diagnostic groups. Within the discordant group, we therefore did not assess differences between PET+/CSF- versus PET-/CSF+ cases due to lack of statistical power. Third, we used four different Aβ PET tracers with slightly different binding properties. Although there seems to be good correspondence between Aβ PET tracers and discordant rates with CSF were within distant range (between 9-17%), some tracer specific effects cannot be excluded.[52] Also, the use of different tracers complicated quantification of PET images, thus Aβ status was solely determined using a binary visual read (following procedures approved by the FDA and EMA). As such, there are no established semiquantitative scales or quantitative thresholds available for our cohort, and we were not able to analyze the frequency and characteristics of borderline PET positive patients. Fourth, we were not able to analyze whether the previously established CSF ratio of tau to amyloid changed discordance patterns.[27] Due to the correction of $A\beta_{42}$ values, to adjust for the longitudinal upward drift observed in our cohort and to use a uniform cut-off value, we applied a different $A\beta_{42}$ cut-off value than previously reported.[19,27] Fifth, amyloid PET visual reads were performed by a single experienced nuclear medicine physician, and we did not specifically examine the reproducibility of these reads. However, in a recent study assessing visual agreement of ^{18}F-flutemetamol PET scans in standardized uptake value ratio (SUVr) and non-displaceable binding potential images(BP_{ND}), the nuclear medicine physician demonstrated good inter-reader agreement with a moderately experienced reader SUVr images and good intra-reader agreement between SUVr and BP_{ND} images.[53] In addition, the agreement between the SUVr and classification (positive/negative) based on quantification was good. Another study assessed inter-reader and inter-methods agreement between three readers using [^{11}C]PIB PET.[54] SUVr images were visually assessed and inter-reader agreement was moderate. Finally, clinical follow-up time was relatively short, and longer follow-up is needed to further characterize the cognitive trajectories of discordant and concordant patients.

FUTURE DIRECTIONS

This study needs to be replicated in an independent sample. Such a study would preferentially be of sufficient size to be able to differentiate PET+/CSF- from PET-/CSF+, include a single Aβ PET tracer to allow PET quantification and take a uniform

approach to handling and analyzing CSF data. Furthermore, identifying the neuroimaging signature (e.g. patterns of gray matter atrophy on structural MRI or glucose hypometabolism on [^{18}F]FDG PET) and neuropathological features of the discordant group could provide insight into the neurobiological mechanisms of Aβ biomarker discrepancies and AD neuropathogenesis.

CONCLUSIONS

In conclusion, we found that patients with discordant Aβ PET and CSF markers were intermediate to concordant-negative and concordant-positive patients in terms of genetic and CSF markers of AD. Discordant biomarkers are not benign in patients without dementia given their higher risk of clinical progression, suggesting that discordant Aβ biomarkers provide important diagnostic and prognostic information in these patients.

Abbreviations: AD, Alzheimer's disease; APOE, Apolipoprotein E; Aβ, Amyloid-β; Aβ42, Aβ 1–42; CSF, Cerebrospinal fluid; MCI, Mild cognitive impairment; MMSE, Mini-Mental State Examination; PET, Positron emission tomography; SCD, Subjective cognitive decline; TMT, Trail Making Test; VAT, Visual Association Test

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Authors' contributions: AdW, JR, RO, and FBo conceived the study, designed the protocol, analyzed/ interpreted data, and drafted the manuscript. JR and AdW performed the statistical analysis. RO, FBo, and PS provided the overall study supervision. CT, MZ, AW, RB, WvdF, PS, and BvB had a major role in the acquisition of data and critically revised and edited the manuscript for intellectual content. All authors read and approved the final version of the manuscript.

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Availability of data and material: The data used in this study are not publicly available but may be provided upon reasonable request.

Ethics approval and consent to participate: The local medical ethics committee of VU University Medical Center has approved a general protocol for biobanking and using the clinical data for research purposes.

Consent for publication: Not applicable.

Competing interests: The authors declare that they have no competing interests.

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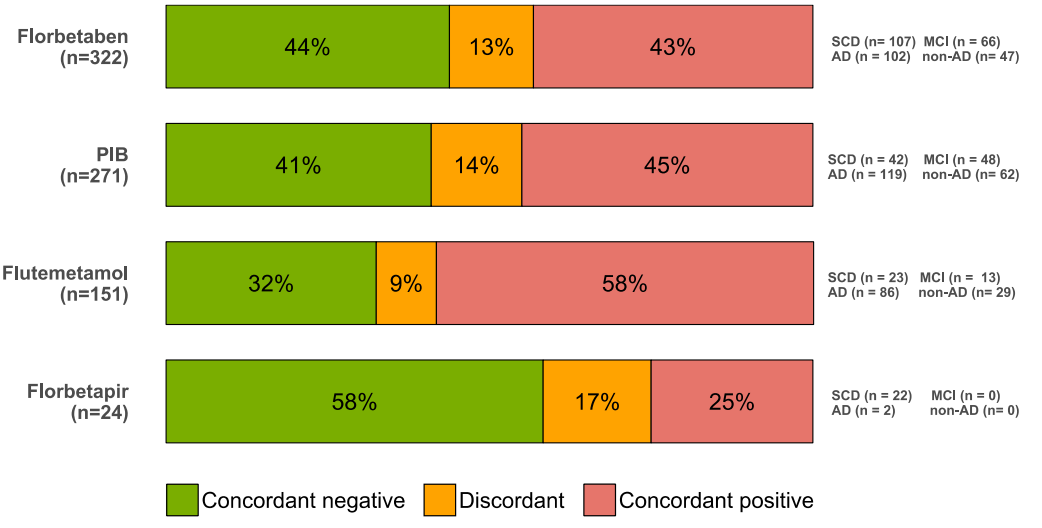
SUPPLEMENTARY DATA



Supplementary Figure 1. Proportions of discordant and concordant patients per Aβ PET tracer

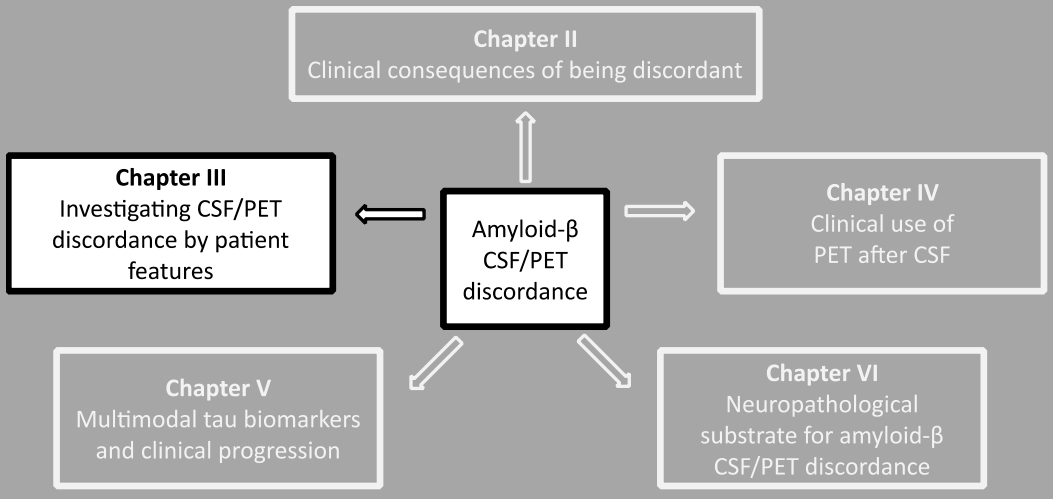
Supplementary Table 1. Proportions of missing neuropsychological test data per domain

Domain	Test	Missing				
		All	PET-/CSF-	Discordant	PET+/CSF+	*P* - value
Global cognition	Mini-Mental State Examination	7.0%	7.7%	7.6%	6.3%	0.486
Memory	Rey Auditory Verbal Learning Test (Dutch) - Visual Association Test	26.5%	21.3%	26.3%	30.3%	<0.001
	Rey Auditory Verbal Learning Test (Dutch) - Total Immediate Recall	24.0%	20.5%	21.4%	27.0%	0.005
	Rey Auditory Verbal Learning Test (Dutch) - Delayed recall	24.4%	20.9%	21.9%	27.4%	0.005
Language	Visual Association Test - naming	26.6%	21.9%	25.4%	30.2%	<0.001
	Category Fluency (animals)	23.2%	20.8%	20.5%	25.5%	0.040
Attention	Trail-Making Test A	24.0%	19.6%	19.2%	28.2%	<0.001
	Digit Span – Forward Condition	26.1%	21.7%	24.1%	29.6%	0.001
	Stroop Test Card I	34.8%	27.9%	34.8%	39.9%	<0.001
	Stroop Test Card II	36.2%	28.7%	35.7%	41.7%	<0.001
Executive	Trail-Making Test B	25.8%	20.2%	21.0%	30.8%	<0.001
	Digit Span – Backward Condition	26.8%	22.3%	25.0%	30.5%	0.001
	Stroop Test Card III	40.7%	31.7%	38.8%	47.6%	<0.001
	Frontal Assessment Battery	29.5%	21.7%	31.7%	34.5%	<0.001
	Controlled Oral Word Association Test (Dutch) - Letter Fluency	31.3%	26.5%	29.5%	35.2%	<0.001
Visuospatial	Visual Object and Space Perception Battery - Incomplete Letters	39.9%	39.0%	40.6%	40.4%	0.824
	Visual Object and Space Perception Battery - Dot Counting	39.5%	38.3%	42.0%	39.8%	0.597
	Visual Object and Space Perception Battery - Number Location	40.2%	37.0%	40.6%	42.5%	0.064

Supplementary Table 2. Longitudinal slopes of cognitive domains in concordant and discordant patients

	Non-demented						Demented					
	CSF-/PET-	p	Discordant	p	CSF+/PET+	p	CSF-/PET-	p	Discordant	p	CSF+/PET+	p
Median (IQR) follow-up time, months	1.9 (1.1-2.4)		1.4 (1.1-2.2)		1.8 (1.1-3.0)		2.0 (1.0-2.8)		1.4 (1.0-2.3)		2.0 (1.1-3.0)	
MMSE (SE)	-0.13 (0.08)	0.19	0.08 (0.15)	1	-0.75 (0.08)[b]	<0.001	-1.40 (0.2)	<0.001	-0.99 (0.27)	<0.001	-0.94 (0.09)	<0.001
Cognitive domains, Z-scores:												
Memory (SE)	-0.04 (0.05)	0.76	-0.03 (0.09)	1	-0.53 (0.05)[b]	<0.001	-0.69 (0.16)	<0.001	-0.23 (0.23)	0.65	-0.55 (0.09)	<0.001
Language (SE)	-0.03 (0.02)	0.39	0.01 (0.04)	1	-0.09 (0.03)	0.001	-0.39 (0.09)	<0.001	-0.13 (0.13)	0.66	-0.32 (0.05)	<0.001
Attention (SE)	-0.07 (0.02)	0.004	0.02 (0.04)	1	-0.10 (0.03)[a]	<0.001	-0.28 (0.05)	<0.001	-0.26 (0.07)	0.001	-0.31 (0.03)	<0.001
Executive (SE)	-0.02 (0.03)	0.77	-0.04 (0.05)	0.95	-0.14 (0.03)	<0.001	-0.49 (0.07)	<0.001	-0.30 (0.11)	0.016	-0.37 (0.04)	<0.001
Visuospatial (SE)	-0.05 (0.04)	0.42	0.18 (0.13)	0.36	-0.10 (0.04)	0.014	-0.66 (0.12)	<0.001	-0.53 (0.18)	0.005	-0.71 (0.07)	<0.001

Abbreviations: IQR, interquartile range; MMSE, Mini-Mental State Examination; SE, standard error. *P* values reported in columns indicate whether the corresponding slope was significantly different from 0.[a] $P < 0.05$; [b] $P < 0.001$


Chapter II
Clinical consequences of being discordant
Chapter III
Investigating CSF/PET discordance by patient features
Amyloid-β CSF/PET discordance
Chapter IV
Clinical use of PET after CSF
Chapter V
Multimodal tau biomarkers and clinical progression
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Neuropathological substrate for amyloid-β CSF/PET discordance

CHAPTER III. PET and CSF amyloid-β status are differently predicted by patient features: Information from discordant cases

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ABSTRACT

Background: Amyloid-β PET and CSF $A\beta_{42}$ yield discordant results in 10-20% of memory clinic patients, possibly providing unique information. Although the predictive power of demographic, clinical, genetic and imaging features for amyloid-positivity has previously been investigated, it is unknown whether these features differentially predict amyloid-β status based on PET or CSF, or whether this differs by disease stage.

Methods: We included 768 patients (subjective cognitive decline (SCD, n=194), mild cognitive impairment (MCI, n=127), dementia (AD and non-AD, n=447) with amyloid-β PET and CSF $A\beta_{42}$ measurement within one year. 97(13%) patients had discordant PET/CSF amyloid-β status. We performed parallel random forest models predicting separately PET and CSF status using 17 patient features (demographics, APOE4 positivity, CSF (p)tau, cognitive performance, and MRI visual ratings) in the total patient group and stratified by syndrome diagnosis. Thereafter, we selected features with the highest variable importance measure (VIM) as input for logistic regression models, where amyloid status on either PET or CSF was predicted by (i) the selected patient feature, and (ii) the patient feature adjusted for the status of the other amyloid modality.

Results: APOE4, CSF tau and p-tau had highest VIM for PET and CSF in all groups. In the amyloid-adjusted logistic regression models, p-tau was a significant predictor for PET-amyloid in SCD (OR=1.02[1.01-1.04], p_{FDR}=0.03), MCI (OR=1.05[1.02-1.07], p_{FDR}<0.01) and dementia (OR=1.04[1.03-1.05], p_{FDR}<0.001), but not for CSF-amyloid. APOE4 (OR=3.07[1.33-7.07], p_{unc}<0.01) was associated with CSF-amyloid in SCD, while it was only predictive for PET-amyloid in MCI (OR=9.44[2.93,30.39], p_{FDR}<0.01). Worse MMSE scores (OR=1.21[1.03-1.41], p_{unc}=0.02) were associated to CSF-amyloid status in SCD, whereas worse memory (OR=1.17[1.05-1.31], p_{FDR}=0.02) only predicted PET positivity in dementia.

Conclusion Amyloid status based on either PET or CSF was predicted by different patient features and this varied by disease stage, suggesting that PET-CSF discordance yields unique information. The stronger associations of both APOE4 carriership and worse memory z-scores with CSF-amyloid in SCD suggests that CSF-amyloid is more sensitive early in the disease course. The higher predictive value of CSF p-tau for a positive PET scan suggests that PET is more specific to AD pathology.

INTRODUCTION

Alzheimer's disease (AD) is characterized by the accumulation of amyloid-β plaques, which has been shown to occur decades before symptom onset.[1,2] Amyloid-β pathology can be detected *in vivo* by positron emission tomography (PET) using amyloid-β radiotracers such as [^{11}C]Pittsburgh compound-B (PIB), [^{18}F]Florbetapir, [^{18}F]Florbetaben or [^{18}F]Flutemetamol allows to directly visualize fibrillary amyloid-β deposits in brain tissue.[3–6] Alternatively, $A\beta_{42}$ levels in cerebrospinal fluid (CSF) reflect the concentration of soluble amyloid-β, which correlates with cerebral amyloid-β depositions.[7] PET and CSF have been included as equal alternatives into diagnostic criteria for both research[2,8,9] and clinical practice,[10–12] although they measure amyloid in different pools (i.e. CSF and cortical brain tissue). In addition, it has been repeatedly shown in memory clinic cohorts that in 10-20% of patients these modalities yield conflicting results.[13–15] In our previous work, we showed that PET/CSF discordance also inflicts patient prognosis and thus has potential clinical consequences.[16] This discordance may include valuable information on underlying clinical or neuropathological differences.[17]

A combination of various patient features has previously been demonstrated to predict amyloid-β positivity based on PET and/or CSF.[18,19] In particular, a combination of demographic information, *APOE* ε4 carriership, neuropsychological tests, and magnetic resonance imaging (MRI) measures was effective in predicting amyloid-β status.[20] Additionally, CSF tau and p-tau have been shown to be predictive of amyloid PET status.[21] So far it has not been investigated whether the predictive ability of patient features for amyloid-β pathology differs when detected by PET or by CSF. We hypothesized that if there are significant differences in the predictive patterns of the two modalities, they must convey partially independent information. Additionally, as it has been suggested that CSF might be able to detect amyloid-β depositions earlier,[22] it is possible that the relative predictive contribution of a patient feature changes throughout the course of Alzheimer's disease. Therefore, in this exploratory study we investigate the unique information provided by the PET-CSF discordant population using the predictive patterns for amyloid PET and CSF in (i) the total patient group and (ii) stratifying by syndrome diagnosis. Exploring this allows us to gain insight in the clinical and neurobiological factors related to discordant results between amyloid-β PET and CSF and ultimately about the underlying neuropathological processes during the disease course of AD.

METHODS

Study Population

We retrospectively included 777 patients, who had visited our tertiary memory clinic between 2005 and 2017 and had undergone both CSF $A\beta_{42}$ analysis and amyloid-β PET within one year. We excluded nine patients that did not pass PET imaging quality control. Patients were screened according to the standardized protocol of the Amsterdam Dementia Cohort.[23,24] This includes a clinical and neuropsychological evaluation, *APOE* genotyping, MR imaging and a lumbar puncture for CSF analysis. Patient diagnosis was determined during a multidisciplinary meeting, according to international guidelines.[10,11,25–33]

Neuropsychological testing

Subjects underwent extensive neuropsychological testing as part of their diagnostic process. Mini-Mental State Examination (MMSE) scores were used to measure global cognition. In addition, five cognitive domains were assessed.[34] We used the visual association test (VAT), total immediate recall, and the Dutch version of the Rey Auditory Verbal Learning test (delayed recall) to assess memory. Language was assessed by VAT naming and category fluency (animals). The Trail-Making Test (TMT) part A, Digit Span forwards and the Stroop test I and II were used for attention. Executive functioning was assessed by TMT B, Digit Span backwards, Stroop test III, the Frontal Assessment Battery, and the Dutch version of the Controlled Oral Word Association Test (letter fluency). Finally, we assessed visuospatial functioning by Visual Object and Space Perception battery: tests incomplete letters, dot counting and number location.

For every test, we derived Z-scores using the mean and standard deviation values from a group of healthy controls (n = 360).[34] TMT-A, TMT-B and Stroop Test scores were log-transformed to account for the non-normal distribution of the data and multiplied by -1 so that lower scores would indicate worse performance. In case TMT B was aborted and TMT A was available (n = 132), we estimated the TMT B score using the multiplication of TMT A score with mean TMT B/A score ratio from the respective diagnostic group.[35] Thereafter, based on available tests we used z-scores to compile a composite score for each of the five cognitive domains.

CSF

CSF was obtained by lumbar puncture between L3/4, L4/5 or L5/S1 intervertebral space, using a 25-gauge needle and a syringe.[36] The samples were collected in polypropylene microtubes and centrifuged at 1800g for 10min at 4°C. Thereafter, the

samples were frozen at -20 °C until manual analyses of Ab_{42}, tau and p-tau were performed using sandwich ELISAs [Innotest assays: β-amyloid1-42, tTAU-Ag and PhosphoTAU-181p; Fujirebio (formerly Innogenetics)] at the Neurochemistry Laboratory of the Department of Clinical Chemistry of VUmc. As the median CSF $Aβ_{42}$ values of our cohort have been gradually increasing over the years,[37] we determined CSF amyloid-β status using $Aβ_{42}$ values that had been adjusted for the longitudinal upward drift. We used a uniform cut-off of 813 pg/mL to dichotomize CSF data.[38]

PET

Amyloid-β PET scanning is not part of standard diagnostic process in the Amsterdam Dementia Cohort. Patients underwent an amyloid-β PET for research purposes in the vast majority[39–44] or otherwise in case of a diagnostic dilemma. Amyloid-β PET scans were performed using the following PET scanners: ECAT EXACT HR+ scanner (Siemens Healthcare, Germany) and Gemini TF PET/CT, Ingenuity TF PET-CT and Ingenuity PET/MRI (Philips Medical Systems, the Netherlands). We included PET scans using four different radiotracers: [^{18}F]Florbetaben[39,44] (n=322, 42%), [^{11}C]PIB[41–43] (n=271, 35%), [^{18}F]Flutemetamol[45] (n=151, 20%), and [^{18}F]Florbetapir[40] (n=24, 3%). PET scans were rated as positive or negative based on visual read by an expert nuclear medicine physician (BvB). PET scans were performed, on average, within 54 (±75) days of the lumbar puncture.

MRI

The acquisition of MRI scans has been extensively described previously.[24] During the period of 2005 to 2017, the following scanners have been used: Discovery MR750 and Signa HDXT (both GE Medical Systems, USA); Ingenuity TF PET/MR (Philips Medical Systems, The Netherlands); Titan (Toshiba Medical Systems, Japan); Magnetom Impact and Sonata (Siemens Healthcare, Germany). The MRI protocol included 3D T1-weighted, T2-weighted, fluid-attenuated inversion recovery (FLAIR), gradient-echo T2* and/or susceptibility weighted imaging sequences. The scans were visually assessed by a neuroradiologist on three different image planes. Parietal atrophy was rated using the posterior cortical atrophy (PCA) scale,[46] medial temporal atrophy using the medial temporal lobe atrophy (MTA) scale[47] and the extent of white matter hyperintensities according to the Fazekas scale.[48] MTA and PCA scores were scored separately for right and left and averaged thereafter. In addition, the scans were assessed for the existence of lacunes and microbleeds.

Patient groups

We stratified the patients based on syndrome diagnosis: subjective cognitive decline (SCD, n=194 (29%)),[49] mild cognitive impairment (MCI, n=127 (17%)), and dementia (n=447 (58%)). Within the dementia group, 309 (69%) patients had the diagnosis of Alzheimer's disease, 66 (15%) a diagnosis within the frontotemporal dementia spectrum, 22 (5%) dementia with Lewy bodies, 6 (1%) vascular dementia and 44 (10%) other dementia syndromes. Patient diagnosis was determined without knowledge of PET or CSF status. To reflect the information provided to the models in our analysis, we present patient group characteristics based on the binarized amyloid-β status on PET and CSF: concordantly positive (PET+/CSF+) or negative (PET-/CSF- for amyloid-β pathology, or discordantly positive amyloid-β status based on PET (PET+/CSF-) or CSF (PET/CSF+).

Statistical Analysis

Statistical analysis was performed using R software (Version 3.4.4).[50] When presenting our study population by binarized PET/CSF status groups, we compared patient features using Chi-squared tests, two samples *t*-tests, Wilcoxon Rank-Sum tests and linear regression models with Bonferroni correction for group-wise testing. Cognitive scores were compared while adjusting for age, sex, education and syndrome diagnosis.

All subsequent analyses were performed in the total patient group as well as in the syndrome diagnosis groups of SCD, MCI and dementia. We first summarized the relative predictive power of every variable in predicting PET and CSF amyloid-β status using random forest modelling. We performed random forest modelling to (i) get an estimate of the predictive power of variables in a setting, where all variables are present in the model (ii) compare the importance of variables between models predicting PET and CSF amyloid-β status and (iii) select patient features for multivariable logistic regression models. As classifier models are affected by missing data, we accounted for missing values using multiple imputations (using the *mice* library[51] including only the 17 predictor variables later used for analysis; with 25 imputations and 5 iterations) (Additional file 1**: Supplementary Table 1)**. For each of the imputed dataset, we ran two conditional random forest models (ntree = 1001, mtry = 5),[52,53] predicting separately PET and CSF status using various patient features associated with Alzheimer's disease.[18–20] As predictors, we selected demographic information (age, sex, education), biomarkers (*APOE* ε4 positivity, CSF tau and p-tau), cognitive measures (MMSE; z-scores for memory, language, attention, executive, visuospatial), and MRI scores (MTA, PCA, Fazekas scale, the presence of lacunes and microbleeds). Accuracy, sensitivity and specificity of the random forest models were evaluated using the mean out-of-bag (OOB) error estimates. Using this method, the performance of

every tree in the random forest model is evaluated on the approximately 37% of observations that are not used for its training, allowing a means to train the model and perform analysis in the same dataset.[54]

We used the area-under-the-curve (AUC)-based permutation variable importance measure (VIM) to estimate the relative predictive power for every patient feature. This measure was selected because of its higher accuracy in datasets with an unbalanced outcome class[55] and we expected this to be especially helpful in the SCD group with a low prevalence of amyloid-β positivity. The AUC-based permutation variable VIM is calculated as follows:

$$\mathrm{VI}_{\mathrm{j}}^{(\mathrm{AUC})} = \frac{1}{\mathrm{ntree}} \sum_{\mathrm{t}=1}^{\mathrm{ntree}} (\mathrm{AUC}_{\mathrm{tj}} - \mathrm{AUC}_{\mathrm{tj}}^{\sim})$$

Where 1) ntree denotes the number of trees in the forest whose OOB observations include observations from both outcome classes 2) $\mathrm{AUC}_{\mathrm{tj}}$ denotes the area under the curve computed in the OOB observations in the selected tree before permuting predictor j and 3) $\mathrm{AUC}_{\mathrm{tj}}^{\sim}$ denotes the area under the curve computed from the OOB observations in tree t after randomly permuting predictor j.[55] As the variable is indirectly dependent on the size of population, these variables cannot be reliably compared between populations of different size. We preferred this VIM measure over several alternative VIM measures, including the Gini imputiry criterion (which might show bias when predictors vary in their number of categories or scale of measurement), the error-rate based permutation mutation (which might falsely identify the importance of highly correlated variables), or error-rate based conditional permutation (which performs best in balanced datasets, while our dataset is unbalanced).[53,55,56]

For the second stage of the analysis, we selected patient features based on their predictive value in the random forest models. Similar to a previous study,[20] we included patient features when their median VIM over the 25 random forests models for predicting either PET or CSF was higher than the median VIM of all the features for the patient group. First, using Wilcoxon signed-rank tests for paired data in 1000x bootstrapped samples with replacement, we compared the VIM of every selected patient feature between the parallel random forest models predicting amyloid-β PET and CSF status. Secondly, to determine the unadjusted predictive power of these patient features, we performed bivariate logistic regression models with either PET or CSF positivity as the outcome and the selected patient features as predictors. Thirdly, to investigate the added predictive value of a patient feature to the other amyloid-β modality, we performed multivariable logistic regression models, with either PET or CSF positivity as the outcome and the selected patient feature with the status of the other amyloid-β modality as predictors. For these models we assumed that if PET and CSF would truly provide equal information about amyloid status, additional patient features should never be significant predictors in these models, as the other amyloid

status would already provide sufficient predictive power. However, if a patient feature added significant information, this would show a stronger association between the feature and the predicted amyloid-β modality.

Finally, as confirmation for our main findings for *APOE* ε4 positivity, CSF tau and p-tau, we compared these multivariable logistic regression models to a univariate logistic regression model, where PET or CSF status was predicted only by the status of the other amyloid modality. We calculated the difference in Akaike information criterion (AIC) between the two models to investigate the change in model fit. A decrease in AIC between models can be interpreted as some (0-2), considerable (4-7) or strong (>10) evidence for gain in model fit in favor of the second model.[57]

We calculated the odds ratios (OR) with corresponding 95% confidence intervals for every patient feature both in the original dataset and in the 25x imputed datasets. Non-overlapping confidence intervals were considered significantly different. We used the False discovery rate (FDR) correction with a significance level of 0.05 to account for multiple testing.[58]

RESULTS

PET/CSF discordance

In total, 32 patients (4%) were discordantly amyloid-β positive based on PET and 65 (8%) based on CSF. The proportion of PET/CSF discordance was 15% in SCD (n= 30), 13% in MCI (n=17) and 11% in dementia (n=50). Of the discordant group, 67% (n=20/30) of SCD, 53% (n=9/17) of MCI and 72% (n=36/50) of dementia were PET-CSF+.

Overview of features

Patient characteristics grouped by PET/CSF status are summarized in **Table 1** and CSF $A\beta_{42}$ levels shown in **Figure 1**. In general, the PET+CSF+ group showed a higher proportion of APOE ε4 carriers, more AD-like CSF markers, MRI features, and lower cognitive scores compared to PET-CSF- group. CSF tau and p-tau were lower in both PET-CSF- and PET-CSF+ groups, compared to PET+CSF- and PET+CSF+. The PET-CSF- group contained a lower proportion of *APOE* ε4 carriers and better cognitive scores than patients in the discordant groups.

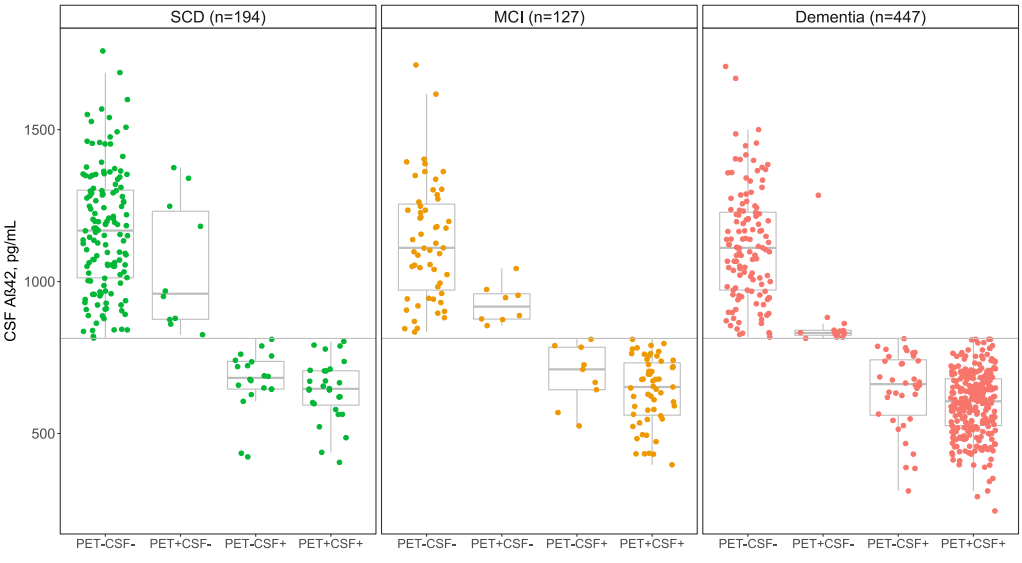

Figure 1. CSF $A\beta_{42}$ values by PET/CSF amyloid status groups in SCD, MCI and dementia

Horizontal line indicates the cut-off of 813 pg/mL used for dichotomization of CSF-amyloid.

Patient feature selection

Out-of-bag accuracy, sensitivity and specificity rates for the random forest models are reported in Additional file 1**: Supplementary Table 2**.

VIM values over the 25 random forest models (one with each set of imputed data) for the total group are shown in **Figure 2a**. *APOE* ε4 positivity was the most important predictor for amyloid-β positivity in the total patient group for both PET and CSF. CSF tau was similarly important when predicting PET or CSF, but CSF p-tau was a more important predictor for PET compared to CSF. Subsequently, we stratified for syndrome diagnosis (**Figure 2b-d**). In SCD, *APOE* ε4 positivity was a stronger predictor for CSF than PET, whereas CSF p-tau was more associated with PET than CSF amyloid-β status. Additionally, MMSE and memory score had a stronger association with CSF than PET. CSF tau was equally important for predicting PET or CSF amyloid-β status. In contrast to the findings in SCD, in MCI, A*POE* ε4 carriership was a stronger predictor for PET than for CSF. Moreover, CSF tau and p-tau were more important for predicting PET than for CSF amyloid-β status. In dementia, CSF p-tau was more predictive of PET than CSF, but CSF tau was a stronger predictor for CSF than for PET amyloid-β status. Both PET and CSF had a strong association to *APOE* ε4 carriership. Finally, visuospatial and memory scores were more important for predicting PET positivity.

Table 1. Patient groups by PET/CSF amyloid status

	PET-CSF-	PET+CSF-	PET-CSF+	PET+CSF+
DEMOGRAPHICS:				
N (%)	315 (41)	32 (4)	65 (8)	356 (46)
Sex, male (%)	211 (67)[D]	17 (53)	41 (63)	192 (54)[A]
Age, years (mean (SD))	62.8 (7.7)	65.0 (7.7)	62.4 (9.0)	63.7 (7.3)
Education (median [IQR])	5 [4, 6]	5 [4, 6]	5 [4, 6]	5 [4, 6]
SYNDROME DIAGNOSIS (%):				
SCD	136 (43)	10 (31)	20 (31)	28 (8)
MCI	55 (18)	8 (25)	9 (14)	55 (15)
AD dementia	28 (9)	11 (34)	17 (26)	253 (71)
non-AD dementia	96 (31)	3 (9)	19 (29)	20 (6)
BIOMARKERS:				
CSF-PET difference, days (mean (SD))	61 (75)	54 (70)	74 (84)	58 (67)
CSF $A\beta_{42}$, pg/mL (median [IQR])	1134 [989, 1275][BCD]	875 [832, 959][ACD]	674 [625, 741][ABD]	615 [537, 688][ABC]
CSF tau, pg/mL (median [IQR])	277 [207, 375][BD]	468 [324, 716][AC]	287 [174, 501][BD]	609 [403, 845][AC]
CSF p-tau, pg/mL (median [IQR])	44 [35, 54][BD]	67 [50, 90][AC]	41 [28, 61][BD]	82 [58, 103][AC]
APOE E4 positivity (%)	84 (28)[BCD]	18 (60)[A]	32 (52)[A]	238 (70)[A]
COGNITION:				
MMSE (mean (SD))	26 (3)[BD]	24 (5)[A]	25 (4)	23 (4)[A]
Memory z-score (mean (SD))	-1.39 (2.27)[BD]	-3.14 (2.73)[A]	-2.20 (2.96)	-3.34 (2.76)[A]
Language z-score (mean (SD))	-0.65 (1.29)	-0.95 (1.48)	-1.44 (2.27)[C]	-1.03 (1.83)[D]
Attention z-score (mean (SD))	-0.69 (1.09)[D]	-0.82 (1.08)	-0.98 (1.02)	-1.10 (1.21)[A]
Executive z-score (mean (SD))	-1.01 (1.38)[D]	-1.39 (1.55)	-1.27 (1.32)	-1.53 (1.40)[A]
Visuospatial z-score (mean (SD))	-0.34 (1.18)[D]	-1.04 (1.90)	-0.90 (1.70)	-1.36 (2.40)[A]
MRI:				
MRI-amyloid difference, days (mean (SD))	16 (50)[C]	35 (60)	44 (78)[AD]	14 (45)[C]
MTA (median [IQR])	0.5 [0.0, 1.0][D]	0.5 [0.0, 1.0]	0.5 [0.0, 1.8]	1.0 [0.5, 1.5][A]
PCA (median [IQR])	1.0 [0.0, 1.1][D]	1.0 [1.0, 1.0]	1.0 [0.0, 1.4][D]	1.0 [1.0, 2.0][AC]
Fazekas (median [IQR])	1.0 [0.0,1.0]	1.0 [0.8, 1.0]	1.0 [0.0, 2.0]	1.0 [0.0, 1.0]
Lacune positivity (%)	14 (6)	0 (0)	7 (11)	17 (7)
Microbleed positivity (%)	31 (13)	4 (15)	4 (7)	54 (21)

Continued from previous page. Education is staged by Verhage classification (1-7). Lacune and microbleed positivity is scored, if at least one is present. MTA - medial temporal lobe atrophy scale. PCA - posterior cortical atrophy scale. A, B, C, D indicate significant difference ($p < 0.05$) from other groups: A - difference from PET-CSF-; B - difference from PET+CSF-; C - difference from PET-CSF+; D - difference from PET+CSF+.

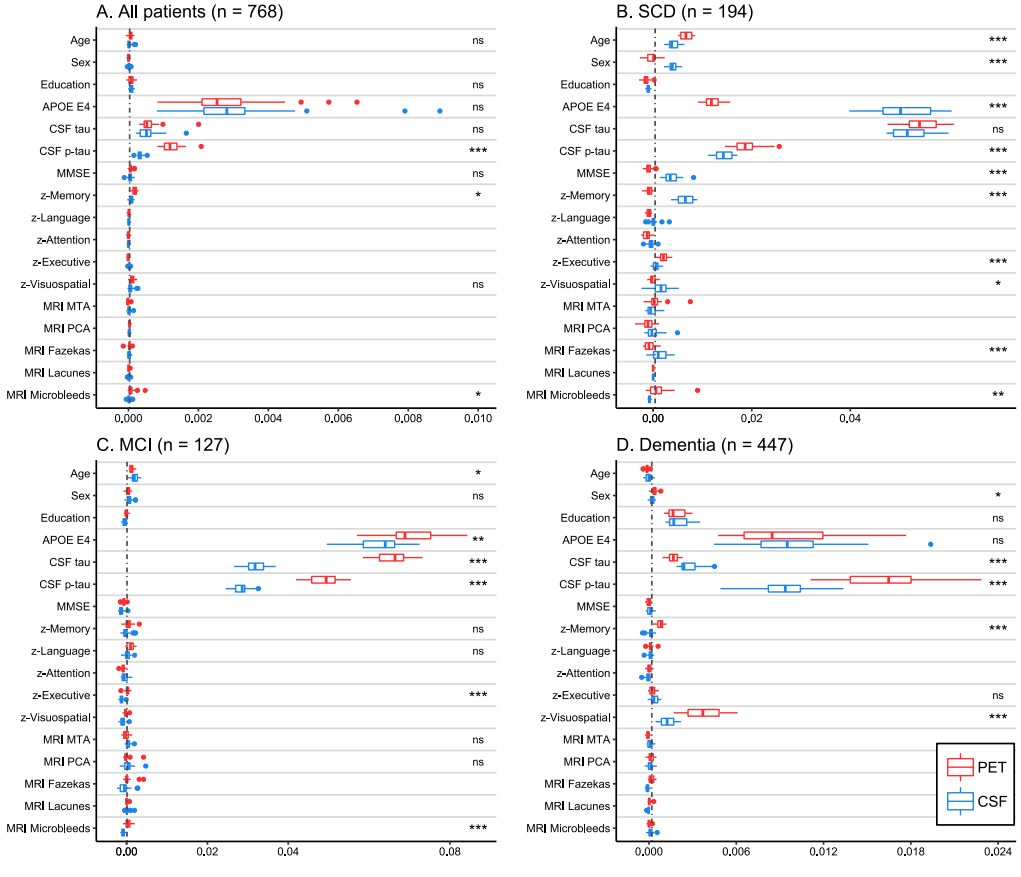

Figure 2a-d. Relative predictive power of patient features for amyloid PET and CSF status

AUC-based variable importance (VIM) from 25 random forest models predicting PET status and 25 models from predicting CSF status are plotted. P-values (*** - p<0.001, ** - p<0.01, * - p<0.05, ns - non-significant) indicate the bootstrapped difference of VIM values between models predicting PET and CSF status using Wilcoxon signed-rank tests.

Additionally, in a subanalysis in the total patient group excluding patients with concordantly negative amyloid status and MCI/dementia, CSF p-tau was the most important predictor for PET but not for CSF (n=589, **Supplementary Figure 1**).

Univariate logistic regression models

We verified the predictive ability of the selected patient features with bivariate logistic regression models for PET and CSF status (**Table 2;** all possible models in **Supplementary Table 3**). The bivariate models largely confirmed the feature selection of the random forest procedure, as *APOE* ε4, CSF tau and CSF p-tau were consistently significant predictors in all groups. In the total group and dementia, most of the patient features selected based on the random forest models were significant predictors.

Amyloid-adjusted multivariable logistic regression models

We investigated the added predictive value of the selected patient features to the other amyloid-β modality with multivariable logistic regression models (odds ratios and p values are shown in **Table 3**; all possible models in **Supplementary Table 4**)**.** In the total group increased levels of CSF p-tau and were more strongly associated with PET than CSF. In SCD, increased levels of CSF p-tau and tau were predictive of only PET, but not CSF positivity. APOE ε4 carriership and lower MMSE scores showed a predictive trend towards amyloid-β status based on CSF, but not on PET. In MCI, a positive PET scan was more strongly predicted by *APOE* ε4, and by increased levels of CSF p-tau and tau. Finally, in dementia, PET status had a stronger association with increased levels of CSF p-tau, tau and with a worse performance in memory, and visuospatial ability than CSF amyloid-β status. *APOE* ε4 carriership was similarly associated with both PET and CSF. No patient feature showed a higher association with CSF in dementia.

AIC change between multivariable and univariate models including amyloid status only

Multivariable logistic regression models including APOE ε4 carriership, CSF tau and CSF p-tau as predictors usually showed significant (>2) decrease of AIC compared to univariate logistic regression models, where PET or CSF status was predicted only by the status of the other amyloid modality (**Table 4**). Overall, differences between change of AIC when predicting PET or CSF were similar to findings from previous random forest and multivariate logistic regression models, indicating consistent results across multiple statistical approaches.

Table 2. Predictive value of patient features for amyloid status based on PET or CSF

		TOTAL					SCD					MCI					DEMENTIA				
					Imputed					Imputed					Imputed					Imputed	
Predictor	Outcome	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR
Age	PET	1.02 (1.00,1.04)			1.02 (1.00,1.04)		1.06 (1.01,1.12)	*		1.06 (1.01,1.12)		0.96 (0.92,1.00)			0.96 (0.92,1.00)						
	CSF	1.01 (0.99,1.03)			1.01 (0.99,1.03)		1.04 (1.00,1.09)			1.04 (1.00,1.09)		0.96 (0.92,1.00)			0.96 (0.92,1.00)						
Sex, F	PET						1.64 (0.81,3.36)			1.64 (0.81,3.36)		2.45 (1.14,5.26)	*		2.45 (1.14,5.26)		1.70 (1.13,2.53)	**	*	1.70(1.13,2.53)	*
	CSF						1.91 (0.99,3.69)			1.91 (0.99,3.69)		2.01 (0.94,4.28)			2.01 (0.94,4.28)		1.40(0.93,2.12)			1.40 (0.93,2.12)	
Education	PET	1.06 (0.94,1.19)			1.07 (0.95,1.20)												1.28 (1.08,1.52)	**	**	1.30 (1.10,1.53)	**
	CSF	1.04 (0.93,1.17)			1.05 (0.94,1.18)												1.29 (1.08,1.54)	**	**	1.30 (1.09,1.54)	**
APOE E4	PET	4.72 (3.46,6.44)	***	***	4.57 (3.34,6.24)	***	2.97 (1.42,6.20)	**	*	2.97 (1.42,6.19)	*	14.55 (6.08,34.82)	***	***	13.43 (5.62,32.11)	***	3.63 (2.39,5.50)	***	***	3.51 (2.30,5.34)	***
	CSF	4.60 (3.36,6.28)	***	***	4.46 (3.26,6.12)	***	3.82 (1.90,7.70)	***	***	3.75 (1.86,7.57)	**	8.28 (3.70,18.54)	***	***	7.76 (3.49,17.27)	***	3.68 (2.39,5.69)	***	***	3.59 (2.33,5.53)	***
CSF tau	PET	1.005 (1.004, 1.006)	***	***	1.005 (1.004, 1.006)	***	1.004 (1.002, 1.006)	***	***	1.004 (1.002, 1.006)	***	1.008 (1.005, 1.011)	***	***	1.008 (1.005, 1.011)	***	1.004 (1.003, 1.005)	***	***	1.004 (1.003, 1.005)	***
	CSF	1.004 (1.003, 1.005)	***	***	1.004 (1.003, 1.005)	***	1.003 (1.002, 1.005)	***	***	1.003 (1.002, 1.005)	**	1.003 (1.002, 1.005)	***	***	1.003 (1.002, 1.005)	***	1.004 (1.003, 1.004)	***	***	1.003 (1.002, 1.004)	***
CSF p-tau	PET	1.05 (1.04,1.06)	***	***	1.05 (1.04,1.06)	***	1.04 (1.02,1.05)	***	***	1.04 (1.02,1.05)	***	1.05 (1.03,1.07)	***	***	1.05 (1.03,1.07)	***	1.05 (1.04,1.06)	***	***	1.05 (1.04,1.06)	***
	CSF	1.04 (1.03,1.04)	***	***	1.04 (1.03,1.04)	***	1.03 (1.01,1.04)	***	***	1.02 (1.01,1.04)	**	1.03 (1.01,1.04)	***	***	1.03 (1.01,1.04)	***	1.04 (1.03,1.05)	***	***	1.04 (1.03,1.05)	***
MMSE	PET	1.20 (1.15,1.25)	***	***	1.19 (1.14,1.24)	***	1.03 (0.89,1.19)			1.02 (0.88,1.18)											
	CSF	1.20 (1.15,1.25)	***	***	1.19 (1.14,1.25)	***	1.15 (1.01,1.31)	*		1.13 (1.00,1.29)											
Memory	PET	1.36 (1.27,1.47)	***	***	1.36(1.27,1.46)	***	1.13 (0.82,1.55)			1.14 (0.83,1.56)		1.26 (1.02,1.57)	*		1.25 (1.00,1.54)		1.20 (1.10,1.30)	***	***	1.20 (1.10,1.31)	***
	CSF	1.32 (1.23,1.42)	***	***	1.32 (1.23,1.42)	***	1.23 (0.92,1.64)			1.22 (0.91,1.62)		1.16 (0.95,1.42)			1.12 (0.92,1.38)		1.14 (1.05,1.24)	**	**	1.15 (1.06,1.26)	**
Language	PET											0.38 (0.18,0.81)	*	*	0.44 (0.21,0.95)						
	CSF											0.71 (0.39,1.27)			0.71 (0.39,1.29)						

Table 2. Continued from previous page

		TOTAL					SCD					MCI					DEMENTIA				
					Imputed					Imputed				Imputed					Imputed		
Predictor	Outcome	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR
Executive	PET						1.02 (0.72,1.45)			1.02 (0.72,1.44)		0.68 (0.45,1.04)			0.70 (0.46,1.05)		0.95 (0.82,1.11)			0.96 (0.82,1.11)	
	CSF						1.05 (0.76,1.44)			1.05 (0.76,1.44)		0.82 (0.55,1.22)			0.83 (0.55,1.24)		0.88 (0.76,1.03)			0.89 (0.76,1.04)	
Visuo-spatial	PET	1.36 (1.22,1.52)	***	***	1.33 (1.19,1.48)	***	0.92 (0.59,1.45)			0.89 (0.56,1.41)							1.30 (1.15,1.49)	***	***	1.25 (1.10,1.43)	**
	CSF	1.38 (1.23,1.55)	***	***	1.34 (1.19,1.50)	***	1.34 (0.93,1.93)			1.35 (0.95,1.93)							1.21 (1.07,1.37)	**	**	1.16 (1.03,1.31)	*
MRI MTA	PET											0.77 (0.48,1.24)			0.75 (0.47,1.20)						
	CSF											1.09 (0.69,1.73)			0.98 (0.62,1.54)						
MRI PCA	PET											0.84 (0.47,1.51)			0.85 (0.48,1.51)						
	CSF											0.74 (0.41,1.32)			0.75 (0.42,1.32)						
MRI Fazekas	PET						0.98 (0.53,1.83)			0.92 (0.50,1.71)											
	CSF						1.38 (0.79,2.41)			1.25 (0.72,2.16)											
MRI microbleeds	PET	1.91 (1.21,3.01)	**	**	1.59 (1.01,2.50)		2.17 (0.75,6.30)			1.84 (0.68,4.99)		1.32 (0.52,3.32)			1.11 (0.47,2.62)						
	CSF	1.51 (0.96,2.39)			1.35 (0.86,2.10)		1.49 (0.52,4.25)			1.32 (0.49,3.52)		1.06 (0.42,2.65)			0.98 (0.41,2.32)						

*** - p<0.001, ** - p<0.01, * - p<0.05. P-values indicate the significance of the patient feature in the model. Uncorrected p-values and corrected p-values are reported per model, additionally corrected p-values for imputed data. False discovery rate (FDR) correction was performed for multiple comparisons. Cognitive scores have been multiplied by -1, therefore lower scores usually indicate higher odds ratios for amyloid positivity.

Table 3. Amyloid-adjusted predictive value of patient features for amyloid status based on PET or CSF

		TOTAL					SCD					MCI					DEMENTIA				
					Imputed					Imputed					Imputed					Imputed	
Predictor	Out-come	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR
Age	PET	1.03 (1.00,1.06)			1.03 (1.00,1.06)		1.04 (0.99,1.10)			1.04 (0.99,1.1)		0.97 (0.91,1.04)			0.97 (0.91,1.04)						
	CSF	0.99 (0.96,1.02)			0.99 (0.96,1.02)		1.02 (0.97,1.07)			1.02 (0.97,1.07)		0.98 (0.92,1.04)			0.98 (0.92,1.04)						
Sex, F	PET						1.17 (0.49,2.77)			1.17 (0.49,2.77)		2.27 (0.75,6.90)			2.27 (0.75,6.90)		1.93 (1.04,3.58)	*		1.93 (1.04,3.58)	
	CSF						1.76 (0.80,3.90)			1.76 (0.8,3.9)		1.11 (0.37,3.35)			1.11 (0.37,3.35)		0.84 (0.44,1.59)			0.84 (0.44,1.59)	
Education	PET	1.06 (0.89,1.27)			1.07 (0.89,1.27)												1.16 (0.9,1.49)			1.18 (0.92,1.51)	
	CSF	1.00 (0.84,1.19)			1.00 (0.84,1.19)												1.15 (0.89,1.50)			1.14 (0.88,1.48)	
APOE E4	PET	2.58 (1.65,4.03)	***	***	2.52 (1.62,3.93)	***	1.54 (0.62,3.78)			1.56 (0.63,3.82)		9.44 (2.93,30.39)	***	**	8.79 (2.72,28.41)	**	2.22 (1.20,4.09)	*	*	2.14 (1.16,3.95)	*
	CSF	2.30 (1.47,3.60)	***	**	2.28 (1.45,3.57)	**	3.07 (1.33,7.07)	**		3.01 (1.3,6.94)		1.85 (0.58,5.92)			1.85 (0.58,5.88)		2.00 (1.06,3.78)	*		2.00 (1.07,3.75)	
CSF tau	PET	1.003 (1.003, 1.004)	***	***	1.003 (1.003, 1.004)	***	1.003 (1.001, 1.005)	**	*	1.003 (1.001, 1.005)	*	1.008 (1.004, 1.012)	***	***	1.008 (1.004, 1.012)	**	1.003 (1.002, 1.004)	***	***	1.003 (1.002, 1.004)	***
	CSF	1.001 (1.000, 1.002)	**	*	1.001 (1.000, 1.002)	*	1.002 (1.000, 1.003)			1.001 (1.000, 1.003)		0.999 (0.997, 1.001)			0.999 (0.997, 1.001)		1.001 (1.000, 1.002)			1.001 (1.000, 1.002)	
CSF p-tau	PET	1.04 (1.03,1.05)	***	***	1.04 (1.03,1.05)	***	1.02 (1.01,1.04)	**	*	1.03 (1.01,1.04)	*	1.05 (1.02,1.07)	***	**	1.05 (1.02,1.07)	**	1.04 (1.03,1.05)	***	***	1.04 (1.03,1.05)	***
	CSF	1.01 (1.00,1.02)	*		1.01 (1.00,1.02)		1.01 (1.00,1.03)			1.01 (0.99,1.02)		0.99 (0.98,1.01)			0.99 (0.98,1.01)		1.01 (1.00,1.02)			1.01 (1.00,1.02)	
MMSE	PET	1.11 (1.05,1.17)	***	**	1.10 (1.04,1.17)	**	0.93 (0.80,1.10)			0.93 (0.79,1.09)											
	CSF	1.10 (1.04,1.16)	**	**	1.10 (1.04,1.16)	**	1.21 (1.03,1.41)	*		1.19 (1.02,1.38)											
Memory	PET	1.22 (1.12,1.34)	***	***	1.22 (1.12,1.33)	***	0.99 (0.69,1.42)			1.01 (0.7,1.46)		1.25 (0.96,1.64)			1.27 (0.97,1.65)		1.18 (1.05,1.32)	**	*	1.17 (1.05,1.31)	*
	CSF	1.09 (1.00,1.19)	*		1.09 (1.01,1.19)		1.23 (0.87,1.75)			1.21 (0.85,1.72)		0.96 (0.71,1.30)			0.92 (0.68,1.25)		1.00 (0.89,1.11)			1.01 (0.91,1.12)	
Language	PET											0.23 (0.08,0.68)	**	*	0.32 (0.10,1.01)						
	CSF											1.59 (0.77,3.27)			1.37 (0.63,2.98)						

Table 3. Continued from previous page

		TOTAL					SCD					MCI					DEMENTIA				
					Imputed					Imputed					Imputed					Imputed	
Predictor	Out-come	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR
Executive	PET						0.99 (0.67,1.48)			0.99 (0.67,1.47)		0.61 (0.33,1.12)			0.62 (0.34,1.14)		1.11 (0.88,1.40)			1.10 (0.87,1.39)	
	CSF						1.05 (0.71,1.55)			1.05 (0.72,1.54)		1.18 (0.64,2.17)			1.17 (0.64,2.15)		0.81 (0.64,1.03)			0.82 (0.64,1.04)	
Visuo-spatial	PET	1.19 (1.03,1.37)	*	*	1.17 (1.03,1.34)	*	0.77 (0.49,1.22)			0.73 (0.46,1.18)							1.32 (1.10,1.59)	**	*	1.28 (1.07,1.53)	*
	CSF	1.20 (1.04,1.39)	*	*	1.16 (1.01,1.34)		1.53 (0.99,2.38)			1.58 (1.01,2.45)							0.99 (0.84,1.17)			0.97 (0.83,1.13)	
MRI MTA	PET											0.55 (0.28,1.05)			0.58 (0.30,1.09)						
	CSF											1.78 (0.87,3.63)			1.52 (0.76,3.03)						
MRI PCA	PET											1.09 (0.48,2.46)			1.11 (0.50,2.48)						
	CSF											0.69 (0.30,1.58)			0.69 (0.31,1.52)						
MRI Fazekas	PET						0.76 (0.36,1.62)			0.7 (0.33,1.5)											
	CSF						1.56 (0.83,2.96)			1.48 (0.78,2.78)											
MRI microbleeds	PET	2.08 (1.07,4.03)	*		1.75 (0.90,3.41)		2.15 (0.60,7.67)			1.94 (0.55,6.86)		1.62 (0.43,6.12)			1.30 (0.36,4.68)						
	CSF	0.89 (0.46,1.73)			0.88 (0.46,1.71)		1.01 (0.29,3.57)			0.91 (0.26,3.17)		0.75 (0.20,2.81)			0.81 (0.22,2.94)						

*** - p<0.001, ** - p<0.01, * - p<0.05. P-values indicate the significance of the patient feature in the model. Uncorrected p-values and corrected p-values are reported per model, additionally corrected p-values for imputed data. False discovery rate (FDR) correction was performed for multiple comparisons. Cognitive scores have been multiplied by -1, therefore lower scores usually indicate higher odds ratios for amyloid positivity

DISCUSSION

We investigated the predictive patterns of various patient features for amyloid-β status based on PET or CSF to determine (i) whether these features have a different association with PET or CSF and (ii) whether this differs per disease stage. We found significant differences in the predictive strength of patient features for amyloid-β status based on PET or CSF. For example, CSF tau, and, especially, CSF p-tau consistently showed a stronger association with amyloid-β status on PET. Additionally, the differential predictive pattern was influenced by the extent of cognitive impairment, as CSF tau was more important in SCD and MCI, while CSF p-tau became more important in the stage of dementia. Moreover, *APOE* ε4 carriership was more predictive towards CSF status in SCD, whereas it was more predictive towards PET in MCI. These findings suggest that PET and CSF do not provide identical information about the stage of Alzheimer's disease.

The idea to study differences in the predictive strength of patient features for PET/CSF amyloid-β status was based on the differences in characteristics of patients with discordant amyloid-β biomarkers, which have been theorized to be caused by various factors. Possible explanations for the discordance include individual variances in CSF

Table 4. Information gain of multivariable logistic regression models compared to univariate logistic regression including only amyloid modalities

		AIC			AIC		
	Predictor	PET ~ CSF	PET ~ CSF + predictor	AIC difference	CSF ~ PET	CSF ~ PET + predictor	AIC difference
Total	APOE E4 positivity	580	533	47	573	531	42
	CSF tau	580	508	71	573	563	10
	CSF p-tau	580	481	99	573	555	17
SCD	APOE E4 positivity	142	138	4	167	152	15
	CSF tau	142	132	10	167	162	5
	CSF p-tau	142	132	10	167	163	4
MCI	APOE E4 positivity	104	86	18	104	100	4
	CSF tau	104	74	30	104	105	-1
	CSF p-tau	104	83	21	104	106	-2
Dementia	APOE E4 positivity	317	295	22	286	267	19
	CSF tau	317	294	23	286	285	2
	CSF p-tau	317	263	54	286	276	10

This table illustrates the change in Akaike Information Criterion (AIC) from the bivariate models including only amyloid modalities (PET ~ CSF and CSF ~ PET) to multivariable models including also an additional predictor. AIC measures model fit and penalizes adding additional predictors. A decrease in AIC between models shows some (0-2), considerable (4-7) or strong (>10) evidence for gain in model fit for the second model.

$A\beta_{42}$ production,[59] the composition of amyloid-β plaques,[60] differences in the structure of Aβ fibrils,[61] or a variety of technical issues,[62,63] including the variability in cut-off values for CSF $A\beta_{42}$.[14] It has also been proposed that in the earliest stages of amyloid-β accumulation CSF $A\beta_{42}$ analysis might be more sensitive, as the decrease in the concentration of soluble isoforms might precede fibrillar amyloid-β plaque deposition detectable by PET.[22] Overall we found significant differences in the relation between amyloid PET and CSF status and other biological variables, such as APOE genotype and (p)tau concentrations. The existence of differing predictive patterns between the two modalities implies that PET/CSF discordance may not only be explained by technical variation, but reflect differences in biological substrate between the modalities. In our previous work, we already showed that PET/CSF discordance has potential clinical consequences.[16] These results could also have an effect for future practice in AD research as well as patient care, as the two modalities are currently used as equal alternatives.[2,11]

Our main finding was that CSF p-tau and tau had a stronger association to amyloid-β based on PET compared to CSF. If we assume that CSF is a more sensitive modality for amyloid-β pathology, then the weaker association with tau could be explained by CSF $A\beta_{42}$ capturing an earlier stage amyloid-β preceding tau pathology. This was reflected by the predictive patterns in the multivariable logistic regression models: when predicting PET status by CSF status, CSF (p)tau adds information about the added burden of disease (including advancing from CSF+PET- to CSF+PET+). When predicting CSF amyloid-β positivity, however, the existence of amyloid-β pathology on PET already provides sufficient predictive power, of subjects already having reached a later stage in amyloid deposition. Overall, although the exact cause of this finding remains unclear, it supports the notion that PET detects more advanced stages of AD pathology, being in accordance with previous work by others.[64] Although CSF tau and p-tau have been shown to be highly correlated,[65] the results of the random forest models imply that CSF tau is more predictive towards amyloid-β pathology in SCD and MCI, whereas CSF p-tau is more predictive in dementia. This finding might be caused by wider neuronal death preceding the release of phosphorylated tau, although previous work seems to suggest that levels of CSF p-tau decrease in the later stages of AD.[66–68] Another possible explanation is that this finding is caused by the greater specificity of p-tau for AD pathology,[69] as our cohort also included amyloid-positive patients diagnosed with non-AD dementia, possibly due to secondary amyloid pathology.

Although we focus on the relative differences between PET and CSF, it should be emphasized that in the majority of cases these two modalities contain similar information. This was demonstrated by our finding that many of the selected patient features had similarly s*ome* predictive power for amyloid-β pathology for both PET and CSF. Of them, the biological factors *APOE* ε4 carriership, CSF tau, and p-tau were

most consistent in having significant predictive ability amyloid-β status irrespective of the modality. These findings are not unexpected, as *APOE* ε4 carriership[18,70,71] and tau pathology[2,72] are widely known to have a strong connection to amyloid-β pathology in Alzheimer's disease. Cognitive measures and MRI visual reads showed overall a smaller predictive value towards amyloid-β status, being in concordance with the theory that they show changes downstream of amyloid and tau pathology.[73]

The main strength of our study is the large number of patients with both amyloid-β modalities from a well-characterized cohort. Nevertheless, there were still a limited number of patients with discordant amyloid status, which could influence the reliability of our findings, especially when performing subgroup analysis. Another limitation is that due to the stratification by syndrome diagnoses, the outcome of amyloid-β positivity was not equally prevalent. Our results in the multivariable logistic regression models might be influenced by the high concordance rate between PET and CSF status, although the results are supported by similar findings in the random forest models and by the decrease in AIC compared to models using only the other amyloid modality as predictors. Additionally, the included patients underwent amyloid-β PET scans with four different radiotracers, allowing for variability in thresholds for amyloid-β positivity. However, this effect is likely reduced by all of the PET scans being visually rated by the same experienced nuclear medicine physician. As continuous measures for PET-imaging were not available, we dichotomized CSF $A\beta_{42}$ values, causing some loss of information, which could influence our results. Finally, this patient group did not have CSF $A\beta_{40}$ values available, which have been shown to correct for the individual variation in the production of amyloid-β.[74,75]

Our findings can be summarized by a hypothetical model highlighting the relative predictive power of patient features towards amyloid-β status based on PET and CSF (**Figure 3**). This model supports previous work, suggesting that CSF might be more sensitive in the early stages of amyloid-β pathology, whereas PET status might be more specific to later stages of amyloid-β accumulation. Although the modalities show similar information in the majority of cases, this could have implications for future research and clinical trials. For example, if aiming to capture the earliest stage of amyloid-β pathology, CSF might be preferred over PET. On the contrary, if high confidence of significant amyloid-β pathology is required, PET could be the modality of choice. Future work in other patient cohorts with a higher number of discordant PET/CSF cases is necessary to replicate these findings.

CONCLUSION

In this exploratory work we demonstrated that although various patient features have general predictive value towards amyloid-β status, there are finer differences revealed

by discordant cases between the predictive pattern for amyloid-β status based on PET and CSF. This indicates that PET-CSF discordance might include valuable information on underlying clinical and neuropathological differences.

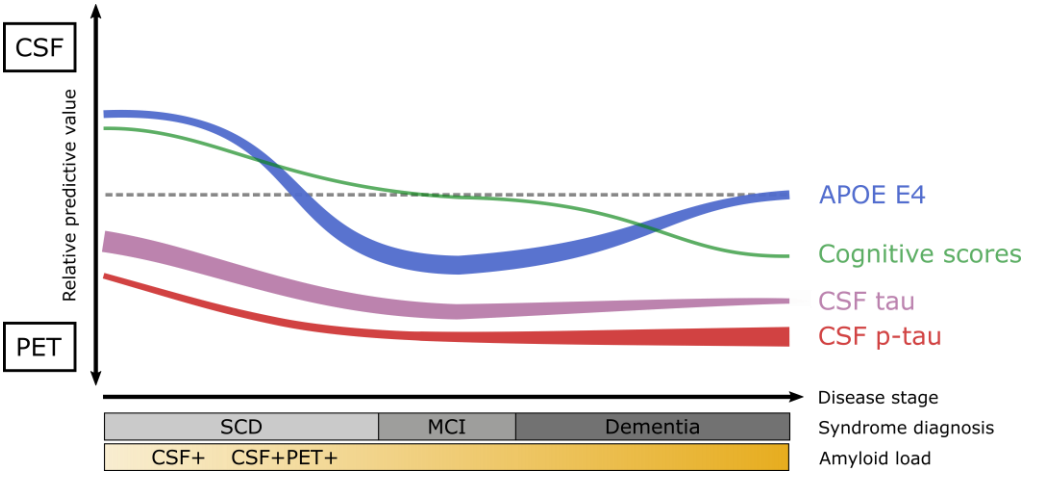

Figure 3. Hypothetical model for relative predictive strength of patient features toward PET and CSF amyloid status

Line location on the y-axis indicates the relative strength of the association between the patient feature and status of the amyloid-β modality. Line thickness indicates the overall predictive strength of the patient feature for amyloid status based on both PET and CSF.

Abbreviations: AD, Alzheimer's disease; AIC, Akaike Information Criterion; AUC, area-under-the-curve; CSF, cerebrospinal fluid; FDR, False discovery rate; MCI, Mild cognitive impairment; MMSE, Mini-Mental State Examination; MRI, Magnetic resonance imaging; MTA, medial temporal lobe atrophy; OOB, out-of-bag; OR, Odds ratio; PCA, P osterior cortical atrophy;PET, Positron emission tomography; SCD. Subjective cognitive decline; TMT, Trail-Making Test; UNC, Uncorrected; VAT, Visual association test; VIM, Variable importance measure

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Authors' contributions: JR, RO, and FBo conceived the study, designed the protocol, analyzed/interpreted data, drafted the manuscript. JR performed statistical analysis. RO, FBo, and PS provided overall study supervision. AdW, CET, MZ, ADW, RB, FBa, WMvdF, PS, BNMvB had a major role in the acquisition of data, and critically revised and edited the manuscript for intellectual content. All authors read and approved the final version of the manuscript.

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SUPPLEMENTARY DATA

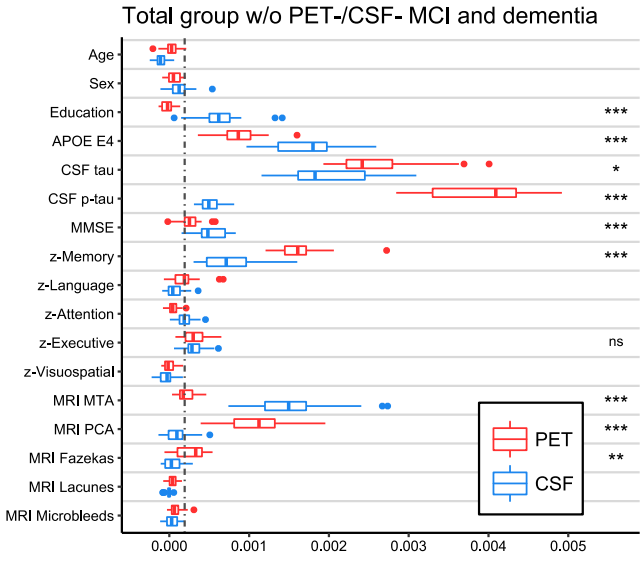
Supplementary Table 1. Proportion of missing values per patient feature

	PET-CSF-	PET+CSF-	PET-CSF+	PET+CSF+
n	315	32	65	356
Age (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Sex (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Education (%)	13 (4.1)	2 (6.2)	3 (4.6)	10 (2.8)
APOE ε4 (%)	12 (3.8)	2 (6.2)	4 (6.2)	14 (3.9)
CSF tau (%)	0 (0.0)	0 (0.0)	1 (1.5)	2 (0.6)
CSF p-tau (%)	2 (0.6)	0 (0.0)	4 (6.2)	0 (0.0)
MMSE (%)	5 (1.6)	1 (3.1)	2 (3.1)	7 (2.0)
Memory z-score (%)	14 (4.4)	3 (9.4)	3 (4.6)	21 (5.9)
Language z-score (%)	17 (5.4)	3 (9.4)	3 (4.6)	25 (7.0)
Attention z-score (%)	15 (4.8)	3 (9.4)	3 (4.6)	22 (6.2)
Executive z-score (%)	6 (1.9)	1 (3.1)	2 (3.1)	12 (3.4)
Visuospatial z-score (%)	23 (7.3)	3 (9.4)	5 (7.7)	36 (10.1)
MRI MTA (%)	68 (21.6)	4 (12.5)	6 (9.2)	88 (24.7)
MRI PCA (%)	91 (28.9)	4 (12.5)	7 (10.8)	93 (26.1)
MRI Fazekas (%)	68 (21.6)	4 (12.5)	2 (3.1)	89 (25.0)
MRI lacunes (%)	76 (24.1)	5 (15.6)	3 (4.6)	95 (26.7)
MRI microbleeds (%)	78 (24.8)	5 (15.6)	10 (15.4)	102 (28.7)

Supplementary Table 2. Out-of-bag accuracy, sensitivity and specificity for random forest models predicting amyloid PET and CSF status

	Outcome	Accuracy %	Sensitivity %	Specificity %
Total	PET	82 (81, 83)	82 (81, 83)	82 (81, 83)
	CSF	78 (77, 78)	81 (80, 82)	74 (73, 75)
SCD	PET	82 (80, 82)	22 (18, 26)	96 (96, 96)
	CSF	79 (77, 80)	31 (27, 36)	94 (94, 95)
MCI	PET	82 (80, 84)	81 (79, 83)	84 (82, 84)
	CSF	72 (69, 75)	74 (70, 77)	70 (65, 75)
Dementia	PET	82 (82, 83)	90 (89, 91)	69 (68, 71)
	CSF	78 (77, 80)	90 (89, 91)	53 (50, 57)

Mean rates with 95% confidence intervals over 25 random forest models are reported.



Supplementary Figure 1. Relative predictive power of patient features for amyloid PET and CSF status when removing PET-CSF- MCI and dementia patients

AUC-based variable importance (VIM) from 25 random forest models predicting PET status and 25 models from predicting CSF status are plotted. P-values (*** - p<0.001, ** - p<0.01, * - p<0.05, ns - non-significant) indicate the bootstrapped difference of VIM values between models predicting PET and CSF status.

Supplementary Table 3. Predictive value of patient features for amyloid status based on PET or CSF

		TOTAL					SCD					MCI					DEMENTIA				
					Imputed					Imputed					Imputed					Imputed	
Predictor	Out-come	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR
Age	PET	1.02 (1.00,1.04)			1.02 (1.00,1.04)		1.06 (1.01,1.12)	*		1.06 (1.01,1.12)		0.96 (0.92,1.00)			0.96 (0.92,1.00)		1.00 (0.97,1.03)			1.00 (0.97,1.03)	
	CSF	1.01 (0.99,1.03)			1.01 (0.99,1.03)		1.04 (1.00,1.09)			1.04 (1.00,1.09)		0.96 (0.92,1.00)			0.96 (0.92,1.00)		0.98 (0.96,1.01)			0.98 (0.96,1.01)	
Sex, F	PET	1.69 (1.26,2.26)	***	***	1.69 (1.26,2.26)	***	1.64 (0.81,3.36)			1.64 (0.81,3.36)		2.45 (1.14,5.26)	*		2.45 (1.14,5.26)		1.70 (1.13,2.53)	**	*	1.70 (1.13,2.53)	*
	CSF	1.55 (1.15,2.07)	**	**	1.55 (1.15,2.07)	**	1.91 (0.99,3.69)			1.91 (0.99,3.69)		2.01 (0.94,4.28)			2.01 (0.94,4.28)		1.40 (0.93,2.12)			1.40 (0.93,2.12)	
Education	PET	1.06 (0.94,1.19)			1.07 (0.95,1.20)		1.10 (0.84,1.46)			1.10 (0.84,1.45)		0.92 (0.69,1.24)			0.91 (0.68,1.22)		1.28 (1.08,1.52)	**	**	1.30 (1.10,1.53)	**
	CSF	1.04 (0.93,1.17)			1.05 (0.94,1.18)		1.05 (0.82,1.35)			1.04 (0.81,1.34)		0.93 (0.69,1.24)			0.94 (0.71,1.26)		1.29 (1.08,1.54)	**	*	1.30 (1.09,1.54)	**
APOE E4	PET	4.72 (3.46,6.44)	***	***	4.57 (3.34,6.24)	***	2.97 (1.42,6.20)	**	*	2.97 (1.42,6.19)	*	14.55 (6.08,34.82)	***	***	13.43 (5.62,32.11)	***	3.63 (2.39,5.50)	***	***	3.51 (2.30,5.34)	***
	CSF	4.60 (3.36,6.28)	***	***	4.46 (3.26,6.12)	***	3.82 (1.90,7.70)	***	**	3.75 (1.86,7.57)	**	8.28 (3.70,18.54)	***	***	7.76 (3.49,17.27)	***	3.68 (2.39,5.69)	***	***	3.59 (2.33,5.53)	***
CSF tau	PET	1.005 (1.004, 1.006)	***	***	1.005 (1.004, 1.006)	***	1.004 (1.002, 1.006)	***	***	1.004 (1.002, 1.006)	***	1.008 (1.005, 1.011)	***	***	1.008 (1.005, 1.011)	***	1.004 (1.003, 1.005)	***	***	1.004 (1.003, 1.005)	***
	CSF	1.004 (1.003, 1.005)	***	***	1.004 (1.003, 1.005)	***	1.003 (1.002, 1.005)	***	**	1.003 (1.002, 1.005)	**	1.003 (1.002, 1.005)	***	***	1.003 (1.002, 1.005)	**	1.004 (1.003, 1.004)	***	***	1.003 (1.002, 1.004)	***
CSF p-tau	PET	1.05 (1.04,1.06)	***	***	1.05 (1.04,1.06)	***	1.04 (1.02,1.05)	***	***	1.04 (1.02,1.05)	***	1.05 (1.03,1.07)	***	***	1.05 (1.03,1.07)	***	1.05 (1.04,1.06)	***	***	1.05 (1.04,1.06)	***
	CSF	1.04 (1.03,1.04)	***	***	1.04 (1.03,1.04)	***	1.03 (1.01,1.04)	***	**	1.02 (1.01,1.04)	**	1.03 (1.01,1.04)	***	***	1.03 (1.01,1.04)	**	1.04 (1.03,1.05)	***	***	1.04 (1.03,1.05)	***
MMSE	PET	1.20 (1.15,1.25)	***	***	1.19 (1.14,1.24)	***	1.03 (0.89,1.19)			1.02 (0.88,1.18)		1.11 (0.95,1.30)			1.12 (0.95,1.31)		1.12 (1.06,1.18)	***	***	1.12 (1.06,1.18)	***
	CSF	1.20 (1.15,1.25)	***	***	1.19 (1.14,1.25)	***	1.15 (1.01,1.31)	*		1.13 (1.00,1.29)		1.02 (0.87,1.20)			1.02 (0.87,1.19)		1.10 (1.04,1.17)	***	**	1.10 (1.04,1.17)	**
Memory	PET	1.36 (1.27,1.47)	***	***	1.36 (1.27,1.46)	***	1.13 (0.82,1.55)			1.14 (0.83,1.56)		1.26 (1.02,1.57)	*		1.25 (1.00,1.54)		1.20 (1.10,1.30)	***	***	1.20 (1.10,1.31)	***
	CSF	1.32 (1.23,1.42)	***	***	1.32 (1.23,1.42)	***	1.23 (0.92,1.64)			1.22 (0.91,1.62)		1.16 (0.95,1.42)			1.12 (0.92,1.38)		1.14 (1.05,1.24)	**	**	1.15 (1.06,1.26)	**
Language	PET	1.10 (1.00,1.20)			1.09 (0.99,1.20)		0.95 (0.56,1.60)			0.94 (0.56,1.57)		0.38 (0.18,0.81)	*		0.44 (0.21,0.95)		0.94 (0.85,1.04)			0.94 (0.85,1.04)	
	CSF	1.20 (1.07,1.34)	**	**	1.19 (1.07,1.33)	**	0.99 (0.62,1.58)			0.99 (0.62,1.56)		0.71 (0.39,1.27)			0.71 (0.39,1.29)		1.00(0.90,1.11)			1.00 (0.90,1.11)	

Supplementary Table 3. Continued from previous page

		TOTAL					SCD					MCI					DEMENTIA				
					Imputed					Imputed					Imputed					Imputed	
Predictor	Out-come	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR
Attention	PET	1.31 (1.14,1.49)	***	***	1.27 (1.12,1.45)	***	1.06 (0.72,1.55)			1.00 (0.69,1.46)		0.57 (0.34,0.94)	*		0.66 (0.41,1.05)		1.03 (0.86,1.23)			1.01 (0.85,1.19)	
	CSF	1.36 (1.19,1.56)	***	***	1.32 (1.16,1.50)	***	1.10 (0.77,1.55)			1.07 (0.76,1.50)		0.86 (0.54,1.37)			0.98 (0.62,1.53)		1.00 (0.83,1.20)			0.97 (0.81,1.16)	
Executive	PET	1.28 (1.15,1.42)	***	***	1.28 (1.15,1.42)	***	1.02 (0.72,1.45)			1.02 (0.72,1.44)		0.68 (0.45,1.04)			0.70 (0.46,1.05)		0.95 (0.82,1.11)			0.96 (0.82,1.11)	
	CSF	1.27 (1.14,1.41)	***	***	1.27 (1.14,1.42)	***	1.05 (0.76,1.44)			1.05 (0.76,1.44)		0.82 (0.55,1.22)			0.83 (0.55,1.24)		0.88 (0.76,1.03)			0.89 (0.76,1.04)	
Visuo-spatial	PET	1.36 (1.22,1.52)	***	***	1.33 (1.19,1.48)	***	0.92 (0.59,1.45)			0.89 (0.56,1.41)		0.72 (0.47,1.08)			0.78 (0.52,1.16)		1.30 (1.15,1.49)	***	***	1.25 (1.10,1.43)	**
	CSF	1.38 (1.23,1.55)	***	***	1.34 (1.19,1.50)	***	1.34 (0.93,1.93)			1.35 (0.95,1.93)		0.98 (0.68,1.42)			1.02 (0.71,1.47)		1.21 (1.07,1.37)	**	**	1.16 (1.03,1.31)	*
MRI MTA	PET	1.27 (1.05,1.53)	*	*	1.25 (1.04,1.50)	*	1.78 (0.90,3.53)			1.56 (0.80,3.04)		0.77 (0.48,1.24)			0.75 (0.47,1.20)		0.77 (0.60,1.00)			0.81 (0.63,1.04)	
	CSF	1.40 (1.16,1.70)	***	**	1.34 (1.11,1.61)	**	1.47 (0.76,2.84)			1.37 (0.72,2.59)		1.09 (0.69,1.73)			0.98 (0.62,1.54)		0.81 (0.62,1.05)			0.84 (0.65,1.09)	
MRI PCA	PET	1.65 (1.32,2.07)	***	***	1.62 (1.30,2.01)	***	1.71 (0.93,3.16)			1.55 (0.87,2.78)		0.84 (0.47,1.51)			0.85 (0.48,1.51)		1.20 (0.88,1.62)			1.18 (0.87,1.59)	
	CSF	1.38 (1.11,1.73)	**	**	1.41 (1.14,1.74)	**	1.07 (0.60,1.90)			1.08 (0.61,1.90)		0.74 (0.41,1.32)			0.75 (0.42,1.32)		0.97 (0.70,1.34)			1.02 (0.75,1.40)	
MRI Fazekas	PET	1.02 (0.82,1.27)			1.01 (0.81,1.25)		0.98 (0.53,1.83)			0.92 (0.50,1.71)		0.83 (0.48,1.42)			0.78 (0.45,1.38)		0.80 (0.60,1.06)			0.83 (0.62,1.11)	
	CSF	1.19 (0.95,1.48)			1.12 (0.90,1.39)		1.38 (0.79,2.41)			1.25 (0.72,2.16)		0.77 (0.45,1.32)			0.74 (0.42,1.30)		0.96 (0.71,1.31)			0.95 (0.70,1.30)	
MRI Lacunes	PET	0.84 (0.43,1.62)			0.85 (0.44,1.65)		0(0,Inf)			0(0,Inf)		0.33 (0.06,1.70)			0.36 (0.07,1.83)		0.72 (0.33,1.59)			0.73 (0.33,1.63)	
	CSF	1.44 (0.73,2.85)			1.25 (0.63,2.46)		6.16 (0.54,69.92)			3.58 (0.35,36.7)		0.31 (0.06,1.63)			0.37 (0.07,2.00)		1.25 (0.51,3.05)			1.06 (0.44,2.58)	
MRI microbleeds	PET	1.91 (1.21,3.01)	**	**	1.59 (1.01,2.50)		2.17 (0.75,6.30)			1.84 (0.68,4.99)		1.32 (0.52,3.32)			1.11 (0.47,2.62)		2.36 (1.16,4.81)	*	*	1.93 (0.93,3.99)	
	CSF	1.51 (0.96,2.39)			1.35 (0.86,2.10)		1.49 (0.52,4.25)			1.32 (0.49,3.52)		1.06 (0.42,2.65)			0.98(0.41,2.32)		1.98 (0.95,4.13)			1.70 (0.81,3.56)	

*** - p<0.001, ** - p<0.01, * - p<0.05. P-values indicate the significance of the patient feature in the model. Uncorrected p-values and corrected p-values are reported per model, additionally corrected p-values for imputed data. False discovery rate (FDR) correction was performed for multiple comparisons. Cognitive scores have been multiplied by -1, therefore lower scores usually indicate higher odds ratios for amyloid positivity.

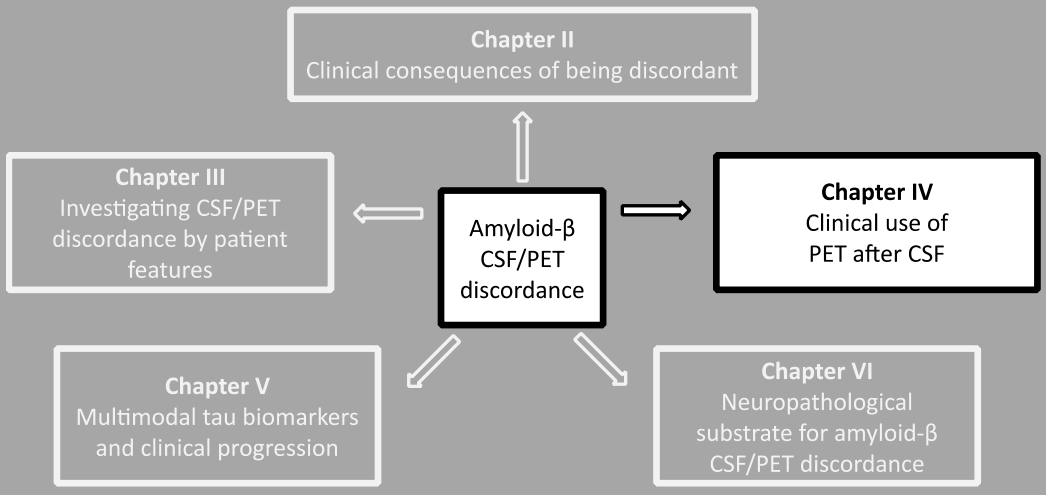
Supplementary Table 4. Amyloid-adjusted predictive value of patient features for amyloid status based on PET or CSF

		TOTAL					SCD					MCI					DEMENTIA				
					Imputed					Imputed					Imputed					Imputed	
Predictor	Out-come	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR
Age	PET	1.03 (1.00,1.06)			1.03 (1.00,1.06)		1.04 (0.99,1.10)			1.04 (0.99,1.10)		0.97 (0.91,1.04)			0.97 (0.91,1.04)		1.03 (0.99,1.07)			1.03 (0.99,1.07)	
	CSF	0.99 (0.96,1.02)			0.99 (0.96,1.02)		1.02 (0.97,1.07)			1.02 (0.97,1.07)		0.98 (0.92,1.04)			0.98 (0.92,1.04)		0.96 (0.93,1.00)			0.96 (0.93,1.00)	
Sex, F	PET	1.57 (1.01,2.44)	*		1.57 (1.01,2.44)		1.17 (0.49,2.77)			1.17 (0.49,2.77)		2.27 (0.75,6.90)			2.27 (0.75,6.90)		1.93 (1.04,3.58)	*		1.93 (1.04,3.58)	
	CSF	1.10 (0.71,1.72)			1.10 (0.71,1.72)		1.76 (0.80,3.90)			1.76 (0.80,3.90)		1.11 (0.37,3.35)			1.11 (0.37,3.35)		0.84 (0.44,1.59)			0.84 (0.44,1.59)	
Education	PET	1.06 (0.89,1.27)			1.07 (0.89,1.27)		1.11 (0.79,1.56)			1.12 (0.79,1.57)		0.95 (0.60,1.50)			0.90 (0.57,1.41)		1.16 (0.90,1.49)			1.18 (0.92,1.51)	
	CSF	1.00 (0.84,1.19)			1.00 (0.84,1.19)		1.00 (0.74,1.35)			0.99 (0.73,1.34)		0.97 (0.61,1.54)			1.02 (0.65,1.60)		1.15 (0.89,1.50)			1.14 (0.88,1.48)	
APOE E4	PET	2.58 (1.65,4.03)	***	***	2.52 (1.62,3.93)	***	1.54 (0.62,3.78)			1.56 (0.63,3.82)		9.44 (2.93,30.39)	***	**	8.79 (2.72,28.41)	**	2.22 (1.20,4.09)	*		2.14(1.16,3.95)	
	CSF	2.30 (1.47,3.60)	***	**	2.28 (1.45,3.57)	**	3.07 (1.33,7.07)	**		3.01 (1.30,6.94)		1.85 (0.58,5.92)			1.85 (0.58,5.88)		2.00 (1.06,3.78)	*		2.00(1.07,3.75)	
CSF tau	PET	1.003 (1.003, 1.004)	***	***	1.003 (1.003, 1.004)	***	1.003 (1.001, 1.005)	**	*	1.003 (1.001, 1.005)	*	1.008 (1.004, 1.012)	***	**	1.008 (1.004, 1.012)	**	1.003 (1.002, 1.004)	***	***	1.003 (1.002, 1.004)	***
	CSF	1.001 (1.000, 1.002)	**	*	1.001 (1.000, 1.002)	*	1.002 (1.000, 1.003)			1.001 (1.000, 1.003)		0.999 (0.997, 1.001)			0.999 (0.997, 1.001)		1.001 (1.000, 1.002)			1.001 (1.000, 1.002)	
CSF p-tau	PET	1.04 (1.03,1.05)	***	***	1.04 (1.03,1.05)	***	1.02 (1.01,1.04)	**	*	1.03 (1.01,1.04)	*	1.05 (1.02,1.07)	***	**	1.05 (1.02,1.07)	**	1.04 (1.03,1.05)	***	***	1.04 (1.03,1.05)	***
	CSF	1.01 (1.00,1.02)	*		1.01 (1.00,1.02)		1.01 (1.00,1.03)			1.01 (0.99,1.02)		0.99 (0.98,1.01)			0.99 (0.98,1.01)		1.01 (1.00,1.02)			1.01 (1.00,1.02)	
MMSE	PET	1.11 (1.05,1.17)	***	**	1.10 (1.04,1.17)	**	0.93 (0.80,1.10)			0.93 (0.79,1.09)		1.22(0.96,1.56)			1.24 (0.97,1.59)		1.10 (1.02,1.19)	*		1.10 (1.01,1.18)	
	CSF	1.10 (1.04,1.16)	**	**	1.10 (1.04,1.16)	**	1.21 (1.03,1.41)	*		1.19 (1.02,1.38)		0.88 (0.69,1.12)			0.87 (0.69,1.11)		1.02 (0.94,1.10)			1.02 (0.94,1.11)	
Memory	PET	1.22 (1.12,1.34)	***	***	1.22(1.12,1.33)	***	0.99 (0.69,1.42)			1.01 (0.70,1.46)		1.25 (0.96,1.64)			1.27 (0.97,1.65)		1.18 (1.05,1.32)	**	*	1.17 (1.05,1.31)	
	CSF	1.09 (1.00,1.19)	*		1.09 (1.01,1.19)		1.23 (0.87,1.75)			1.21 (0.85,1.72)		0.96 (0.71,1.30)			0.92 (0.68,1.25)		1.00 (0.89,1.11)			1.01 (0.91,1.12)	
Language	PET	0.95 (0.85,1.07)			0.95 (0.84,1.07)		0.91 (0.45,1.86)			0.91(0.46,1.80)		0.23 (0.08,0.68)	**		0.32 (0.10,1.01)		0.90 (0.79,1.01)			0.89 (0.79,1.01)	
	CSF	1.24 (1.08,1.43)	**	*	1.23 (1.07,1.42)	*	1.03 (0.58,1.82)			1.02(0.59,1.78)		1.59 (0.77,3.27)			1.37 (0.63,2.98)		1.12 (0.95,1.32)			1.13 (0.96,1.34)	

Supplementary Table 4. Continued from previous page

		TOTAL					SCD					MCI					DEMENTIA				
					Imputed					Imputed					Imputed					Imputed	
Predictor	Out-come	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR	Odds ratio (95% CI)	p unc	p FDR	Odds ratio (95% CI)	p FDR
Attention	PET	1.10 (0.91,1.34)			1.09 (0.90,1.32)		1.00 (0.65,1.52)			0.96 (0.63,1.45)		0.38 (0.18,0.80)	*		0.43 (0.21,0.86)		1.07 (0.81,1.40)			1.07 (0.82,1.39)	
	CSF	1.27 (1.03,1.55)	*		1.24(1.02,1.50)		1.10 (0.71,1.70)			1.09 (0.72,1.66)		1.80 (0.88,3.68)			1.87 (0.93,3.77)		0.95 (0.72,1.26)			0.9 2(0.70,1.21)	
Executive	PET	1.17 (1.00,1.37)			1.16 (1.00,1.36)		0.99 (0.67,1.48)			0.99 (0.67,1.47)		0.61 (0.33,1.12)			0.62 (0.34,1.14)		1.11 (0.88,1.40)			1.10 (0.87,1.39)	
	CSF	1.12 (0.96,1.31)			1.13 (0.97,1.32)		1.05 (0.71,1.55)			1.05 (0.72,1.54)		1.18 (0.64,2.17)			1.17 (0.64,2.15)		0.81 (0.64,1.03)			0.82 (0.64,1.04)	
Visuo-spatial	PET	1.19 (1.03,1.37)	*	*	1.17 (1.03,1.34)		0.77 (0.49,1.22)			0.73 (0.46,1.18)		0.55 (0.31,0.96)	*		0.61 (0.36,1.03)		1.32 (1.10,1.59)	**	*	1.28 (1.07,1.53)	
	CSF	1.20 (1.04,1.39)	*	*	1.16 (1.01,1.34)		1.53 (0.99,2.38)			1.58 (1.01,2.45)		1.63 (0.93,2.83)			1.53 (0.89,2.63)		0.99 (0.84,1.17)			0.97 (0.83,1.13)	
MRI MTA	PET	1.00 (0.76,1.31)			1.02 (0.78,1.33)		1.68 (0.77,3.68)			1.48 (0.66,3.32)		0.55 (0.28,1.05)			0.58 (0.30,1.09)		0.79 (0.55,1.15)			0.81 (0.56,1.17)	
	CSF	1.37 (1.06,1.77)	*	*	1.30 (1.01,1.68)		1.15 (0.53,2.51)			1.12 (0.51,2.43)		1.78 (0.87,3.63)			1.52 (0.76,3.03)		0.96 (0.67,1.38)			0.99 (0.69,1.41)	
MRI PCA	PET	1.71 (1.25,2.33)	***	**	1.66 (1.22,2.26)	**	1.87 (0.93,3.76)			1.74 (0.88,3.43)		1.09 (0.48,2.46)			1.11 (0.50,2.48)		1.50 (0.97,2.31)			1.42 (0.93,2.17)	
	CSF	0.95 (0.70,1.29)			0.96 (0.72,1.30)		0.78 (0.39,1.56)			0.78 (0.39,1.57)		0.69 (0.30,1.58)			0.69 (0.31,1.52)		0.74 (0.47,1.14)			0.79 (0.51,1.21)	
MRI Fazekas	PET	0.82 (0.61,1.11)			0.84 (0.62,1.14)		0.76 (0.36,1.62)			0.70 (0.33,1.50)		0.98 (0.46,2.08)			0.95 (0.45,2.02)		0.68 (0.46,1.00)			0.72 (0.48,1.07)	
	CSF	1.35 (1.00,1.81)			1.26 (0.94,1.70)		1.56 (0.83,2.96)			1.48 (0.78,2.78)		0.78 (0.37,1.64)			0.76 (0.36,1.61)		1.28 (0.85,1.94)			1.23 (0.81,1.87)	
MRI Lacunes	PET	0.47 (0.20,1.09)			0.51 (0.22,1.20)		0(0,Inf)			0(0,Inf)		0.54 (0.06,4.90)			0.53 (0.07,4.09)		0.44 (0.16,1.20)			0.47 (0.17,1.33)	
	CSF	2.42 (1.01,5.78)	*		2.03 (0.85,4.86)		12.35 (1.06,143.8)	*		7.61 (0.69,84.47)		0.47 (0.05,4.24)			0.58 (0.07,4.96)		2.2 8(0.73,7.12)			1.89 (0.60,5.95)	
MRI microbleeds	PET	2.08 (1.07,4.03)	*		1.75 (0.90,3.41)		2.15 (0.60,7.67)			1.94 (0.55,6.86)		1.62 (0.43,6.12)			1.30 (0.36,4.68)		2.24 (0.82,6.12)			1.87 (0.69,5.09)	
	CSF	0.89 (0.46,1.73)			0.88 (0.46,1.71)		1.01 (0.29,3.57)			0.91 (0.26,3.17)		0.75 (0.20,2.81)			0.81 (0.22,2.94)		1.08 (0.38,3.09)			1.04 (0.38,2.88)	

*** - p<0.001, ** - p<0.01, * - p<0.05. P-values indicate the significance of the patient feature in the model. Uncorrected p-values and corrected p-values are reported per model, additionally corrected p-values for imputed data. False discovery rate (FDR) correction was performed for multiple comparisons. Cognitive scores have been multiplied by -1, therefore lower scores usually indicate higher odds ratios for amyloid positivity.


Chapter II
Clinical consequences of being discordant
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CHAPTER IV. Why is amyloid-β PET requested after performing CSF biomarkers?

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ABSTRACT

Background: Amyloid-β positron emission tomography (PET) and cerebrospinal fluid (CSF) $A\beta_{42}$ are considered interchangeable for clinical diagnosis of Alzheimer's disease.

Objective: To explore the clinical reasoning for requesting additional amyloid-β PET after performing CSF biomarkers.

Methods: We retrospectively identified 72 memory clinic patients who underwent amyloid-β PET after CSF biomarkers analysis for clinical diagnostic evaluation between 2011 and 2019. We performed patient chart reviews to identify factors which led to additional amyloid-β PET. Additionally, we assessed accordance with appropriate-use-criteria (AUC) for amyloid-β PET.

Results: Mean patient age was 62.0 (SD=8.1) and mean MMSE was 23.6 (SD=3.8). CSF analysis conflicting with the clinical diagnosis was the most frequent reason for requesting an amyloid-β PET scan (n=53, 74%), followed by incongruent MRI (n=16, 22%), unusual clinical presentation (n=11, 15%) and young age (n=8, 11%). An amyloid-β PET scan was rarely (n=5, 7%) requested in patients with a CSF Aβ+/tau+ status. Fifteen (47%) patients with a post-PET diagnosis of AD had a predominantly non-amnestic presentation. In n=11 (15%) cases, the reason that the clinician requested amyloid-β was not covered by AUC. This happened most often (n=7) when previous CSF analysis did not support current clinical diagnosis, which led to requesting amyloid-β PET.

Conclusion: In this single-center study, the main reason for requesting an amyloid-β PET scan after performing CSF biomarkers was the occurrence of a mismatch between the primary clinical diagnosis and CSF Aβ/tau results.

INTRODUCTION

Two methods are currently employed in the clinic to capture *in vivo* amyloid-β pathology, a pathological hallmark of Alzheimer's disease (AD).[1,2] $A\beta_{42}$ levels in cerebrospinal fluid (CSF) reflect the soluble amyloid-β pool that has been shown to correlate with amyloid-β depositions in the brain.[3] Alternatively, amyloid-β positron emission tomography (PET) can be employed to directly visualize parenchymal fibrillary amyloid-β depositions.[4] Although CSF and PET yield conflicting results in 10-20% of patients,[5–7] they are nonetheless considered interchangeable for clinical use.[8]

In our center, all patients are offered CSF biomarker analysis. However, despite the availability of CSF biomarkers, occasionally amyloid-β PET-scans are requested. The diagnostic value of amyloid-β PET to a standard dementia screening has been established in many studies,[9–11] but few studies have included subgroups of people with available CSF biomarkers. Reported reasons for performing amyloid-β PET in such cases included incongruent CSF biomarkers in patients with suspicion of AD or atypical clinical presentation.[12,13] We aimed to elucidate this practice by exploring the clinical reasoning for requesting amyloid-β PET after CSF biomarkers were disclosed, and to characterize the population that received amyloid-β PET after CSF examination.

Additionally, appropriate use criteria (AUC) for amyloid-β PET have been published to support the implementation of clinical amyloid-β PET, advocating use in three groups most likely to benefit: patients with an atypical clinical presentation or mixed etiology, persistent unexplained mild cognitive impairment (MCI), and unexplained dementia in young patients.[14] As a secondary goal, we aimed to compare our clinical practice against current amyloid-β PET AUC.

METHODS

Patient inclusion

We identified 209 cases from the Amsterdam Dementia Cohort with a [^{11}C]-Pittsburgh Compound B (PIB) PET scan between 2011 and 2019 (**Figure 1**). We excluded n=85 cases who underwent PIB-PET for research purposes, n=4 cases with CSF analysis performed after amyloid-β PET and n=2 cases with unknown CSF disclosure. Although lumbar puncture (LP) is offered to all patients visiting our center, it was not performed for n=46 patients, most often because LP was either not successful (n=14, 30%), not possible (n=13, 28%), or refused by the patient (n=12, 26%). Finally, we included 72 cases with a clinically requested amyloid-β PET scan performed after CSF biomarkers examination for our analysis.

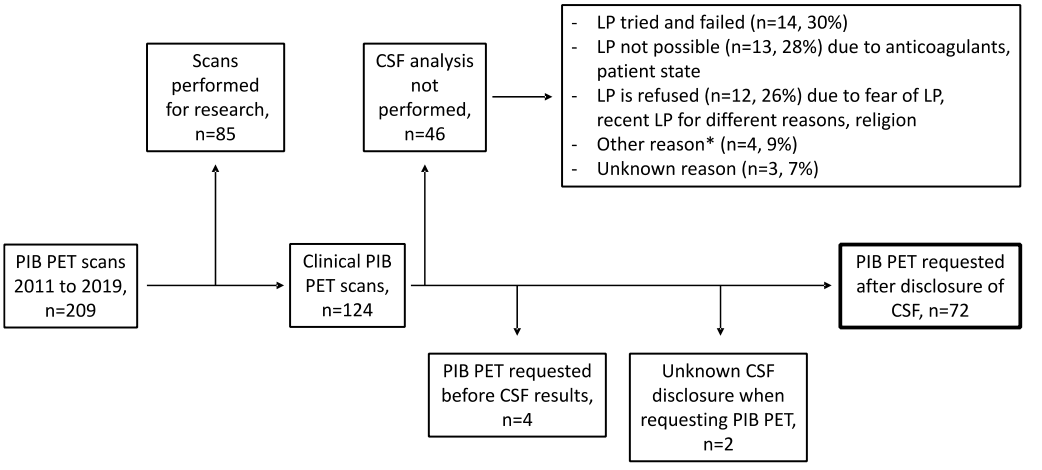


Figure 1. Flowchart for patient inclusion

*Other reasons include normal pressure hydrocephalus, increased certainty received from amyloid-β PET imaging, and imaging having a greater influence on convincing patients. Abbreviations: CSF - cerebrospinal fluid; LP – lumbar puncture; PIB PET – Positron emission tomography with ^{11}C-Pittsburgh compound B.

Cerebrospinal fluid

CSF was obtained by lumbar puncture (LP), using a 25-gauge needle and a syringe.[15] Samples were collected in polypropylene collection tubes and centrifuged at 1800g for 10min at 4°C. Thereafter, samples were frozen at -20 °C until routine biomarker analysis. Manual analyses of $A\beta_{42}$, total tau and phosphorylated tau (p-tau) were performed using sandwich ELISAs (Innotest assays: β-amyloid1-42, tTAU-Ag and PhosphoTAU-181p; Fujirebio) in the Neurochemistry Laboratory of the Department of Clinical Chemistry of Amsterdam UMC. In a few cases, CSF analysis was performed using automated assays for $A\beta_{42}$ (n=9), t-tau (n=1), and p-tau (n=1), due to change in routine methods (Elecsys CSF, Roche Diagnostics GmbH).[16] Additionally, in n=12 cases analyses were performed in the Department of Laboratory Medicine in Radboud UMC prior to referral to our center.

The clinical cut-off values for CSF $A\beta_{42}$ have repeatedly been changed over the years due to the gradual upward drift of median CSF $A\beta_{42}$ values observed in our cohort, possibly due to changes in ELISA kits and/or calibration data that are influenced by the presence of $A\beta_{42}$ aggregates.[17] In order to pool all available CSF values (both local and external) in relation to different cut-offs, we created standardized values by calculating, per patient, the percentage of the CSF value relative to its concurrent cut-off. For example, a value of 150 would represent a normal CSF $A\beta_{42}$ being 50% higher than the cut-off, whereas a value of 80 represented a pathologically decreased CSF $A\beta_{42}$ being 20% below the cut-off value.

Positron emission tomography

Amyloid-β PET is not part of the standard diagnostic process in the Amsterdam Dementia Cohort, therefore most of the amyloid-β PET scans in our center are performed for research purposes. We only included scans with [^{11}C]-PIB, as clinically requested PET scans in our center are routinely performed using [^{11}C]-PIB as the radiotracer. These scans were performed using the following PET scanners: ECAT EXACT HR+ scanner (Siemens Healthcare, Germany) and Gemini TF PET/CT or Ingenuity TF PET-CT (Philips Medical Systems, the Netherlands). PET scans were performed within a median of 140 [IQR=67, 260] days after the lumbar puncture. PET scans were rated as positive or negative based on visual read by an expert nuclear medicine physician.[11] Although intra-rater agreement was not available for this sample, in previous work using [^{11}C]-PIB PET, our nuclear medicine physician showed excellent (Fleiss k = 0.88) and good to moderate (Fleiss k = 0.59 and 0.68) inter-reader agreement for standardized uptake value (SUV), SUV ratio and non-displaceable binding potential images, respectively.[18]

Magnetic resonance imaging (MRI)

MRI was performed as described previously.[19] The scans were visually assessed by a neuroradiologist on three different image planes for posterior cortical atrophy (PCA),[20] medial temporal atrophy (MTA),[21] and global cortical atrophy (CGA),[22] which were thereafter age-normalized.[23] The extent of white matter hyperintensities was rated according to the Fazekas scale.[24] Additionally, the scans were assessed for the existence of lacunes and microbleeds. An external scan was used in n=19 cases, and MRI was not available in n=2 cases (n=1 with available computed tomography [CT]).

Neuropsychological testing

Patients underwent extensive neuropsychological testing as part of their diagnostic process. We used Mini-Mental State Examination (MMSE) scores to measure global cognition.

Additionally, we derived z-scores of various neuropsychological tests using the mean and standard deviation values from a group of healthy controls (n = 360), whose mean age was 57.8 (standard deviation [SD]=8.3) and mean MMSE was 28.2 (SD=1.9). Thereafter, we compiled composite scores for five cognitive domains (memory, language, attention, executive and visuospatial).[25]

Reasons for requesting amyloid-β PET

Our main objective was to explore the clinical reasoning for requesting an amyloid-β PET after disclosure of CSF biomarkers. Therefore, JR and FBo performed patient chart reviews to retrieve the clinical reasoning for requesting the amyloid-β PET scan. Patients were divided into two groups (AD vs non-AD) based on the most likely etiological diagnosis prior to performing a PIB PET scan. For both diagnostic groups we listed characteristics that were recorded as not compatible with the current etiological diagnosis, therefore leading to additional amyloid-β PET. Listed reasons included incongruent findings from biomarkers (CSF, imaging, EEG) or patient history and presentation, as well as other supporting factors such as age, patient wish and implementation of a new CSF assay. For example, in the AD group we labelled CSF as a reason for additional amyloid-β PET when a normal CSF analysis or a CSF analysis with isolated low $A\beta_{42}$ or high tau/p-tau did not support the clinical diagnosis. Similarly, we labelled MRI findings as a reason for additional amyloid-β PET in the non-AD group when a normal MRI or pronounced hippocampal atrophy decreased confidence in the current clinical diagnosis.

Accordance to amyloid-β PET appropriate use criteria

Previously published appropriate use criteria support amyloid-β imaging in case of (i) progressive unexplained MCI (ii) possible AD with atypical or etiologically mixed presentation and (iii) progressive dementia at an early age, usually defined as below the age of 65.[14] Based on examining patient charts, we determined for each case accordance with the PET appropriate use criteria.

Patient population

For all patients we determined an initial available etiological diagnosis as the first diagnosis, the first available diagnosis after amyloid-β PET as the last diagnosis, and diagnostic change as the difference between the two. In n=23 (32%) cases CSF analysis was performed prior to referral to our center (n=14 with Aβ+/tau- or Aβ-/tau+ based on CSF) and n=3 (4%) patients had undergone a previous amyloid-β PET scan.

We present our patient population by the binarized status based on CSF $A\beta_{42}$ and total tau. We chose to use CSF total tau instead of p-tau in order to closely resemble clinical decision-making. Our data showed that in case of dubious diagnosis, increased levels of either CSF total tau or p-tau facilitated further diagnostics and there were more patients with an isolated increase of CSF tau (n=8) than p-tau (n=3). CSF tau and p-tau status were identical in n=61 (85%) of patients.

Statistical Analysis

Statistical analysis was performed using R software (Version 3.4.4).[26–29] We compared patient features using Chi-squared tests, two samples *t*-tests, Wilcoxon Rank-Sum tests and linear regression models. Cognitive scores were compared while adjusting for age, sex, and education.

RESULTS

Demographics

We included n=5 (7%) patients with subjective cognitive decline (SCD), n=3 (4%) whose symptoms were mainly associated with a psychiatric condition, n=16 (22%) with MCI and n=48 (67%) with dementia. The average age in our patient cohort was 62.0 (standard deviation [SD]=8.1), n=46 (64%) of patients were male and average MMSE was 23.6 (SD=3.8) (**Table 1**). Most patients where a clinical PIB-scan was requested were Aβ-/tau- (n=25, 35%), Aβ-/tau+ (n=23, 32%) or Aβ+/tau- (n=19, 26%), while only a minority 5 (7%) had an Aβ+/tau+ status based on CSF (**Figure 2**). In total, n=34 (47%) patients were amyloid-positive based on PET (compared to n=24 (33%) based on CSF $A\beta_{42}$). Amyloid-β CSF and PET status were discordant in n=32 (44%) cases. Amyloid-β PET positivity was lower in the Aβ-/tau- group (n=6, 24%) compared to the Aβ-/tau+ (n=15, 65%; p=0.01) and Aβ+/tau- (n=11, 58%; p=0.048) groups. We found no significant differences in cognitive scores and MRI measures between the groups.

Reasons for amyloid-β PET after CSF

To explore the clinical reasoning for amyloid-β PET, patients were divided into two groups (AD, n=41 and non-AD, n=31) based on the most likely etiological diagnosis prior to performing a PIB PET scan. More than one reason for amyloid-β PET was reported for n=33 (46%) cases. Conflicting information from CSF analysis (either not supporting the current diagnosis or with discordant Aβ/tau status) was the most frequent reason for requesting an amyloid-β PET scan (n=53/72; 74%), being more prevalent in patients with the main suspected etiological diagnosis of AD (n=36/41, 88%) than in patients with a non-AD suspected etiological diagnosis (n=17/31, 55%, p=0.004) (**Figure 3**). Other factors contributing to the request of a clinical amyloid-β PET scan after CSF included MRI not supporting the clinical diagnosis (n=16/72, 22%), unusual clinical presentation (n=11/72, 15%) and young age (n=8/72, 11%). In some cases (n=5/72, 7%, all with Aβ+/tau-), inexperience in interpreting the results of a new CSF $A\beta_{42}$ assay (Elecsys CSF, Roche Diagnostics GmbH) contributed to diagnostic

Table 1. Patient population stratified by binarized CSF $A\beta_{42}$ and tau status

	TOTAL	Normal CSF	Conflicting CSF Aβ and tau		AD-like CSF
		Aβ-/tau-	Aβ-/tau+	Aβ+/tau-	Aβ+/tau+
n (%)	72	25 (35)	23 (32)	19 (26%)	5 (7)
Sex, male (%)	46 (64)	17 (68)	14 (61)	12 (63)	3 (60)
Age (mean (SD))	62.0 (8.1)	63.9 (6.3)	62.9 (9.5)	60.5 (6.4)	54.5 (11.2)
Education (median [IQR])	5 [5, 6]	5 [5, 6]	5 [5, 6]	5 [5, 6]	5 [4, 6]
APOE ε4 carriership (%)	33 (55)	14 (64)	11 (55)	8 (50)	0/2 (0)
Change in diagnosis (%)	37 (51)	20 (80)[BCD]	11 (48)[A]	5 (26)[A]	1 (20)[A]
CSF as a reason for amyloid-β PET (%)	53 (74)	15 (60)	18 (78)	17 (89)	3 (60)
Amyloid-β PET positivity (%)	34 (47)	6 (24)[BC]	15 (65)[A]	11 (58)[A]	2 (40)
PET according to AUC (%)	61 (85)	22 (88)	19 (83)	15 (79)	5 (100)
CSF-PET time difference, days (median [IQR])	140 [67, 260]	140 [75, 261]	162 [78, 304]	124 [61, 204]	109 [34, 260]
MRI as a reason for amyloid-β PET (%)	16 (22)	6 (24)	3 (13)	5 (26)	2 (40)
MTA positivity (%)	32 (46)	14 (56)	8 (40)	8 (42)	2 (40)
PCA positivity (%)	26 (54)	10 (59)	8 (67)	7 (47)	1 (25)
GCA positivity (%)	22 (46)	8 (47)	5 (42)	8 (53)	1 (25)
Fazekas positivity (%)	9 (13)	3 (12)	5 (23)	1 (5)	0 (0)
Lacune positivity (%)	4 (6)	1 (4)	1 (5)	2 (11)	0 (0)
Microbleed positivity (%)	5 (8)	0 (0)	3 (14)	2 (11)	0 (0)
MMSE (mean (SD))	23.6 (3.8)	22.8 (3.6)	23.9 (4.1)	24.4 (3.9)	23.7 (2.5)
Memory z-score (mean (SD))	-3.20 (2.87)	-3.41 (2.22)	-3.19 (4.04)	-2.98 (2.30)	-2.74 (1.14)
Language z-score (mean (SD))	-1.13 (1.39)	-1.30 (1.84)	-1.09 (1.27)	-0.87 (0.79)	-1.81 (1.47)
Attention z-score (mean (SD))	-1.10 (1.09)	-1.25 (1.01)	-0.95 (1.11)	-0.92 (1.14)	-2.57 (0.08)
Executive z-score (mean (SD))	-1.56 (1.41)	-1.82 (1.32)	-1.61 (1.69)	-1.05 (1.13)	-2.07 (1.17)
Visuospatial z-score (mean (SD))	-0.90 (1.42)	-1.02 (1.98)	-0.75 (1.00)	-0.77 (0.78)	-2.07 (1.00)

Education is staged by Verhage classification (1-7).[32] MRI scans were regarded (i) medial temporal atrophy (MTA)-positive if the left-right averaged MTA≥1 for a patient under the age of 65, or ≥1.5 for patient age between 65 and 75 (ii) posterior cortical atrophy (PCA)-positive if the left-right averaged PCA≥1 for a patient under the age of 65 (iii) global cortical atrophy (GCA)-positive if the GCA≥1 for a patient under the age of 65 [23]. Cognitive domain z-scores were derived using the mean and standard deviation values from a group of healthy controls. A, B, C, D indicate difference ($p < 0.05$) from other groups: A - difference from Aβ-/tau-; B - difference from Aβ-/tau+; C - difference from Aβ+/tau-; D - difference from Aβ+/tau+.

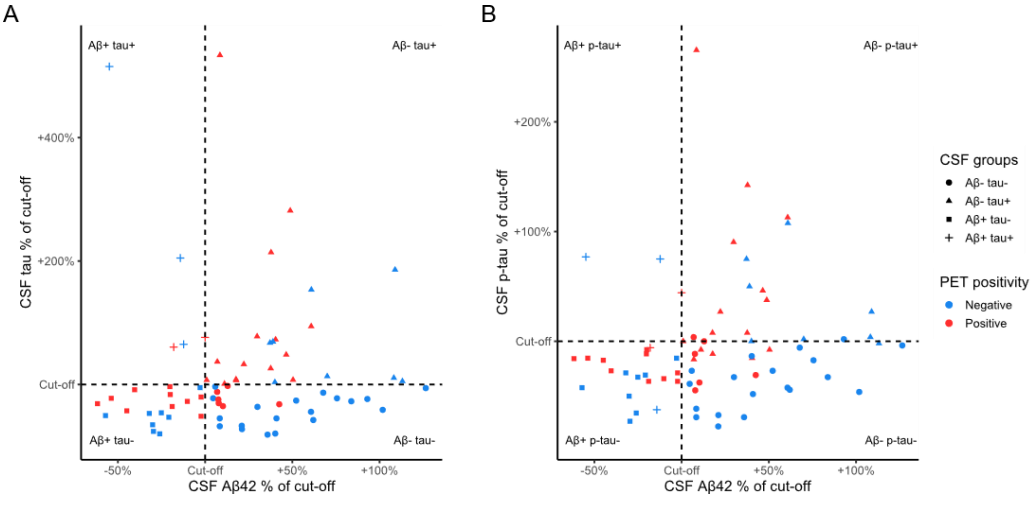


Figure 2. CSF $A\beta_{42}$ and tau / p-tau values relative to their cut-offs

We present standardized CSF values, created by calculating the percentage of the CSF value relative to its concurrent cut-off. Values of <100% represent pathologically decreased CSF $A\beta_{42}$ and values of >100% indicate pathologically increased CSF tau (A) and p-tau (B).

uncertainty leading to PIB PET scan. A reason for requesting a PET scan was not recorded in the patient chart for n=2/72 (3%) cases.

Accordance to amyloid-β PET AUC

In most cases (n=61, 85%), amyloid-β PET scans were performed in compliance with the AUC. Our clinical practice was not covered by the AUC in n=7 (n=3 amyloid-negative based on PET) as the clinical findings were suspicious of amnestic AD, but conflicting information from CSF (n=6) or a previously negative amyloid-β PET combined with normal CSF (n=1) led to an amyloid-β PET scan. Finally, an amyloid-β PET scan was requested for n=3 patients without objective cognitive decline, who had decreased $A\beta_{42}$ in the CSF analysis, lowering diagnostic confidence; and for n=1 patient with a known PSEN1 mutation to define the stage of pathological disease progression.

Change in diagnosis

Of the n=42 patients with AD as their initial etiological diagnosis, n=15 (36%) had a predominant non-amnestic presentation (either language-AD (n=6), visuospatial (n=5),

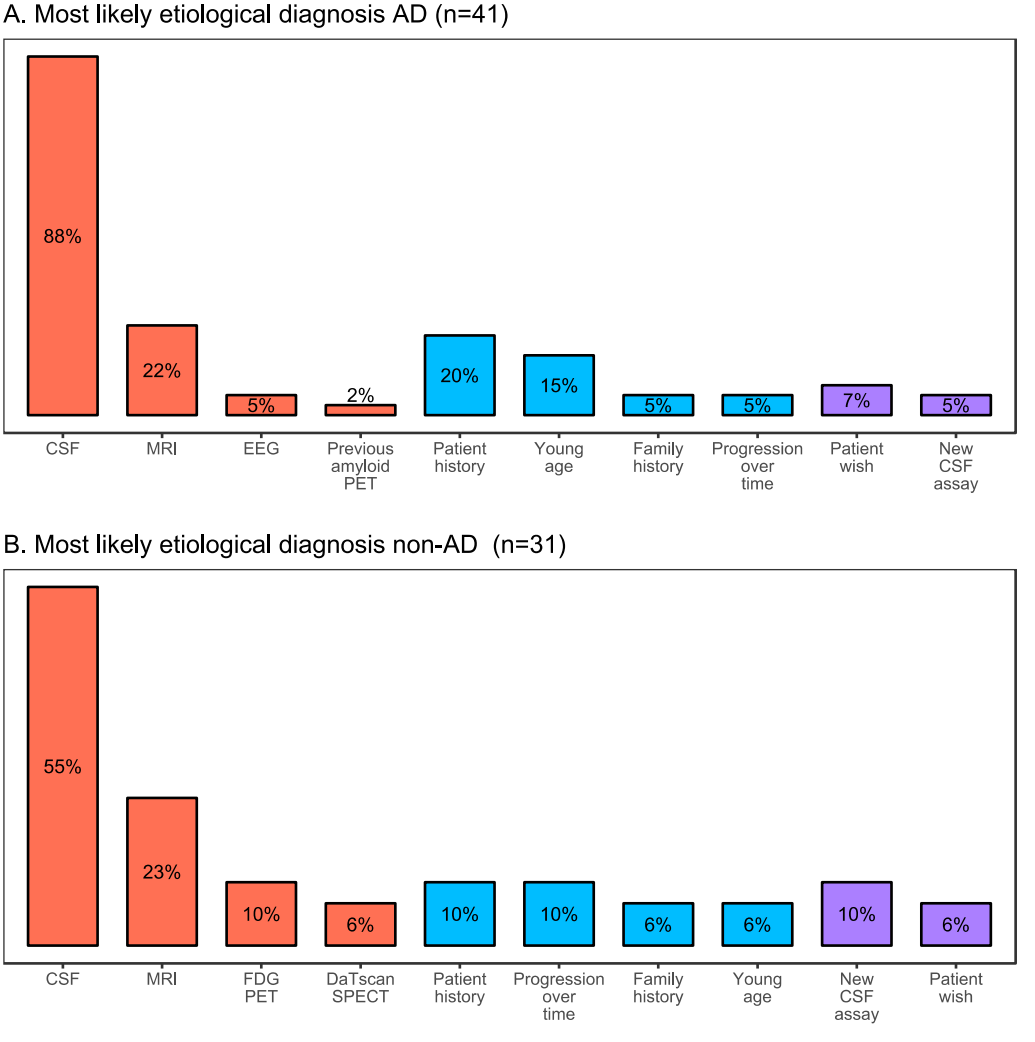


Figure 3. Clinical reasons for requesting additional amyloid-β PET after CSF

Patients are grouped based on most likely diagnosis prior to an amyloid-β PET scan. For both diagnosis groups we list characteristics that were recorded as being not compatible with the current main diagnosis, therefore leading to additional amyloid-β PET imaging. Reasons for the amyloid-β PET scan are grouped as biomarkers (red), patient history and presentation (blue) and external (purple). More than one reason is possible per patient. Abbreviations: CSF – cerebrospinal fluid; MRI – magnetic resonance imaging; EEG – electroencephalography; PET – positron emission tomography; FDG – [^{18}F]fluorodeoxyglucose, SPECT – single-photon emission computed tomography.

behavioral/dysexecutive (n=3) or corticobasal syndrome (n=1)) (**Figure 4**). Likewise, in patients with a final diagnosis of AD, about half (n=15/32, 44%) had a non-amnestic presentation. Twenty-two patients (52%) with an initial clinical diagnosis of AD had

amyloid-β positivity based on PET and n=11 (26%) based on CSF $A\beta_{42}$. Of the patients with AD as the final etiological diagnosis, n=32 (100%) had amyloid-β pathology based on PET and 13 (41%) based on CSF $A\beta_{42}$.

Overall, change in diagnosis occurred in n=37 (51%) of cases. Diagnosis changed more often in the Aβ-/tau- group (n=20, 80%) compared to the Aβ-/tau+ (n=11, 48%; p=0.04), Aβ+/tau- (n=5, 26%; p<0.01), and Aβ+/tau+ (n=1, 20%; p=0.03) groups.

Final diagnoses of the five cases in the CSF Aβ+/tau+ group were (i) early-onset AD (age: 48 years) with a negative family history (ii) autoimmune encephalitis (iii) corticobasal syndrome due to AD (iv) logopenic variant primary progressive aphasia with a previously negative amyloid-β PET scan and (v) a suspected genetic variant of frontotemporal dementia (FTD). To further illustrate the diagnostic process, we present a small case series including one patient from each of the four CSF Aβ/tau groups (**Figure 5**).

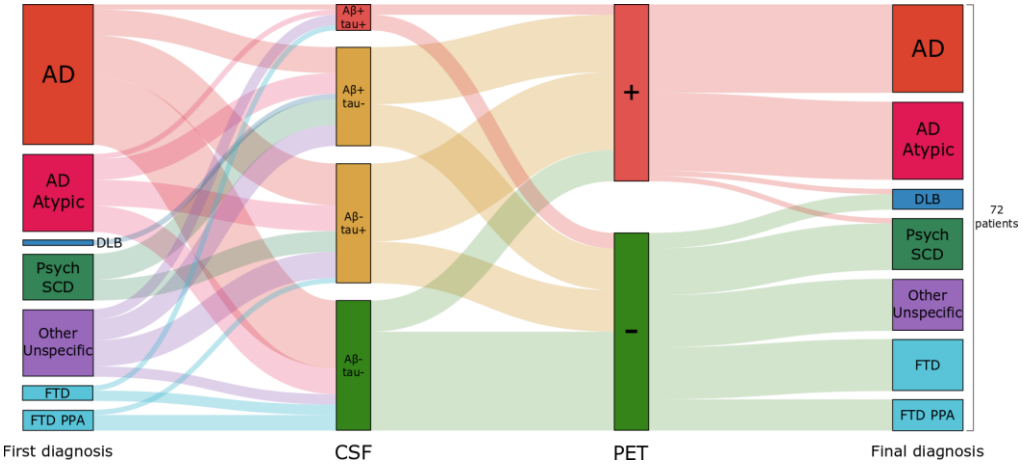


Figure 4. Etiological diagnosis in relation to CSF Aβ/tau status and amyloid-β PET

A Sankey diagram showing (i) the distribution of baseline diagnoses to groups based on CSF Aβ/tau status (ii) the percentage of amyloid-β PET positivity by CSF Aβ/tau groups and (iii) the correlation of final diagnosis to amyloid-β PET positivity. DLB – dementia with Lewy bodies; Psych – psychiatric disorder, SCD – subjective cognitive decline, FTD – frontotemporal dementia, PPA – primary progressive aphasia.

DISCUSSION

We investigated the clinical reasoning behind requesting an amyloid-β PET scan after disclosure of CSF biomarkers in a clinical cohort. Our main finding was that in most cases CSF biomarkers conflicting with the clinical diagnosis contributed to diagnostic

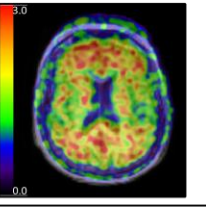
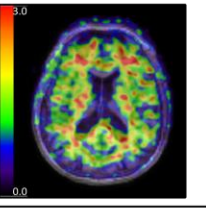
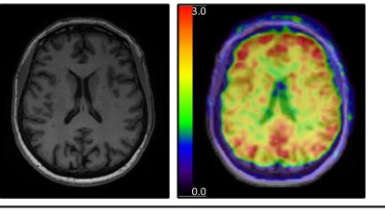
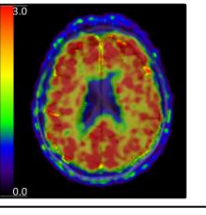
First Diagnosis	CSF (% of cut-off)	Clinical history & Rationale for amyloid-β PET	Amyloid-β PET scan	Last Diagnosis
AD	N N N 143% 68% 69% $A\beta_{42}$ tau p-tau	- 72 y/o woman with MCI / beginning dementia (memory, behaviour) - Family history: five siblings and father with dementia - Clinically suspicious for familial AD, but conflicting CSF leads to PET - Clinical rationale not covered by the amyloid-β PET AUC *Image: [11C]PIB PET scan showing diffuse uptake in grey matter, in accordance with a positive scan.*		AD
AD	N ↑ ↑ 122% 133% 127% $A\beta_{42}$ tau p-tau	- 72 y/o man with progressive memory loss clinically fitting AD - MRI showed evident hippocampal atrophy with MTA grade 3/3, and parietal atrophy (PCA grade 2/2; not shown) - Conflicting CSF with normal Aβ42 led to PIB PET (negative) - Final diagnosis was an atypical presentation of frontotemporal dementia - Clinical rationale not covered by the PET AUC *Image: [11C]PIB PET showing no evident tracer uptake in grey matter, in accordance with a negative scan*		FTD
AD	↓ N N 90% 73% 66% $A\beta_{42}$ tau p-tau	- 54 y/o woman with progressive memory loss - Clinically MCI most probably due to AD - CSF analysis was performed externally 2 months prior to referral - Conflicting CSF with normal tau values, young age, and a normal MRI scan led to requesting additional PIB PET *Left: MRI T1 sequence, showing no evident atrophy; Right: [11C]PIB PET scan showing diffuse uptake in grey matter, in accordance with a positive scan.*		AD
AD atypical	↓ ↑ ↑ 100% 176% 144% $A\beta_{42}$ tau p-tau	- 60 y/o man with dementia - Myoclonus, a possible alien limb syndrome and neglect on the left side - Patient was diagnosed with corticobasal syndrome most likely due to AD - Previously, DLB had been suspected due to hallucinations and fluctuations during an infection; Datscan was performed and was rated as normal (not shown) - A marginally decreased CSF Aβ42 and unclear clinical findings led to requesting additional amyloid-β PET *Image: [11C]PIB PET scan showing diffuse uptake in grey matter, in accordance with a positive scan.*		AD atypical

Figure 5. Four case reports illustrating the clinical reasoning for requesting an additional amyloid-β PET

uncertainty, which led to the request of an amyloid-β PET scan to support the clinical diagnostic process. This was reinforced by the observation that an additional amyloid-β PET scan was rarely requested in patients with a CSF Aβ+/tau+ status. Second, we found that an amyloid-β PET was requested in patients, that were relatively young, often had an atypical presentation of AD and often showed a change in diagnosis. Third, we observed that although amyloid-β PET scans were usually requested according to the AUC, our clinical practice was not wholly covered by these criteria, as it was often driven by inconclusive CSF biomarker results. Our results support previous work that CSF biomarkers that conflict with the clinical diagnosis often lead to additional amyloid-β PET.[12,13]

In our cohort, an amyloid-β PET was most often requested due to inconclusive results from the CSF biomarkers. This occurred in cases when diagnostic confidence was low due to inconclusive CSF Aβ/tau status, or when a clinical diagnosis of AD was contradicted by a non-pathologic CSF analysis, which is largely in agreement with previous findings.[13] In fact, there were few patients with CSF Aβ+/tau+ status in our cohort, indicating that patients with a clinical suspicion of AD supported by low $A\beta_{42}$ and high tau in the CSF analysis usually do not need further confirmation with amyloid-β PET. This is also evident in the current guidelines for both clinical practice[8] and research,[1] which advocate CSF and PET as parallel options to support the diagnosis of Alzheimer's disease. However, our results show that in complicated cases clinicians valued the information from an additional amyloid-β PET.

Although clinical diagnosis conflicting with CSF biomarkers contributed to requesting amyloid-β PET scans in most patients, overall clinical rationale was more complex. This was illustrated by a variety of other factors, often in combination with each other, that decreased clinical diagnostic confidence and led to additional amyloid-β PET imaging. Most prominently, incongruent imaging (MRI, FDG PET, DaTscan SPECT) findings or an unusual clinical presentation contributed to decreased confidence in the clinical diagnosis despite CSF findings. The added value of amyloid-β PET imaging, in particular in atypical clinical presentations of AD, has also been shown previously.[12,30] Our results also support younger patient age being a factor for requesting an amyloid-β PET scan, as indicated in the PET AUC.[14] This is related to younger patients more often having a non-amnestic clinical presentation, in addition to the diagnosis of AD being rare and potentially having a higher impact at a younger age. Finally, we observed that a clinician's decision can also be influenced by external reasons, such as patient wish or decreased confidence in CSF results due to the initiation of a new CSF assay. It is also possible that clinicians as well as patients might also be inclined to have more confidence in PET imaging due to the visual aspects of a PET scan, and that clinicians' biases and prior experiences might play a role when deciding whether to use additional amyloid-β PET diagnostics.

Amyloid-β PET was usually, but not always, requested in accordance with the PET appropriate use criteria.[14] Some differences between clinical practice and the AUC were not unexpected as the AUC were designed to build an initial framework for clinical amyloid-β PET, and were also published during the time course of our study. When clinical practice was not covered by the AUC, a PIB scan was requested in patients with no objective cognitive decline due to decreased $A\beta_{42}$ values in the CSF, or due to decreased diagnostic confidence arising from inconclusive or normal CSF biomarker (or prior amyloid-β PET) results in patients with a clinical syndrome suggestive of AD. Although the recently published AUC for CSF also include performing CSF analysis in SCD,[31] neither the AUC for CSF nor the AUC for PET describe the diagnostic setting, where information about amyloid-β status is already available. As amyloid-β biomarkers are increasingly integrated into clinical practice, the number of such cases is likely to increase over time. The value of an additional amyloid-β PET is likely highest in patients with a CSF analysis conflicting with the clinical diagnosis of AD, as a negative amyloid-β PET scan can refute the diagnosis.[11] In cases with a non-AD diagnosis combined with a decreased $A\beta_{42}$ and/or an increased tau in the CSF, the added value of an amyloid-β PET scan is hindered by the possibility of amyloid-β as a secondary pathology, especially in older populations. Therefore, our results combined with previous work from other centers[12,13] suggest a group of patients (i.e. clinically diagnosed with AD without an AD-like CSF biomarker signature) might benefit from being included in updated amyloid PET AUC. However, these findings must be confirmed by larger prospective multi-center studies.

The main strength of the present study is the description of the clinical practice in a tertiary memory clinic, where both CSF biomarkers are regularly used for clinical practice and there is good access to amyloid-β PET if needed. By excluding cases where an amyloid-β PET scan was performed due to involvement in research, we were able to minimize the bias caused by research and to concentrate on the clinical decision-making process. In addition, our study has some limitations. Composition of our sample resulted in some inherit biases, caused by the infrastructure of our memory clinic. For example, all patients in our center are offered CSF biomarkers analysis, many patients (often with prior CSF analysis available) are referred to us due to a diagnostic dilemma, and referred patients are generally relatively young. Additionally, all patients are assessed by five neurologists in our center, who might share similar views on the application of biomarkers. While these sample characteristics might reduce overall generalizability, we believe our findings are likely to be generalizable to other memory clinic settings where CSF analysis is commonly used and represent a relevant clinical question. Additionally, due to the retrospective nature of the study, some of the data were retrieved from patient charts. In cases of incomplete or ambiguous descriptions, some degree of subjective judgement on the part of the investigators was unavoidable. Our center has also been involved in several amyloid-

β PET studies,[9–11] which recruited patients from clinical practice. Therefore, our cohort may have missed cases where amyloid-β PET was deemed clinically useful from that period of time. Finally, due to the longitudinal upward drift of the median CSF $A\beta_{42}$ values in our centers, the cut-off values for CSF $A\beta_{42}$ in our center have been changing over time.[17] Therefore, it is feasible that the CSF $A\beta_{42}$ cut-offs did not always best represent the underlying amyloid-β status.

To conclude, we presented data from a single memory clinic where CSF biomarkers are commonly used. During the period of our study, the main reason for requesting an amyloid-β PET scan was the occurrence of a mismatch between the primary clinical diagnosis and CSF Aβ/tau results. Future work is necessary to confirm similar clinical reasoning in other cohorts and to consider whether such practice should be represented in guidelines.

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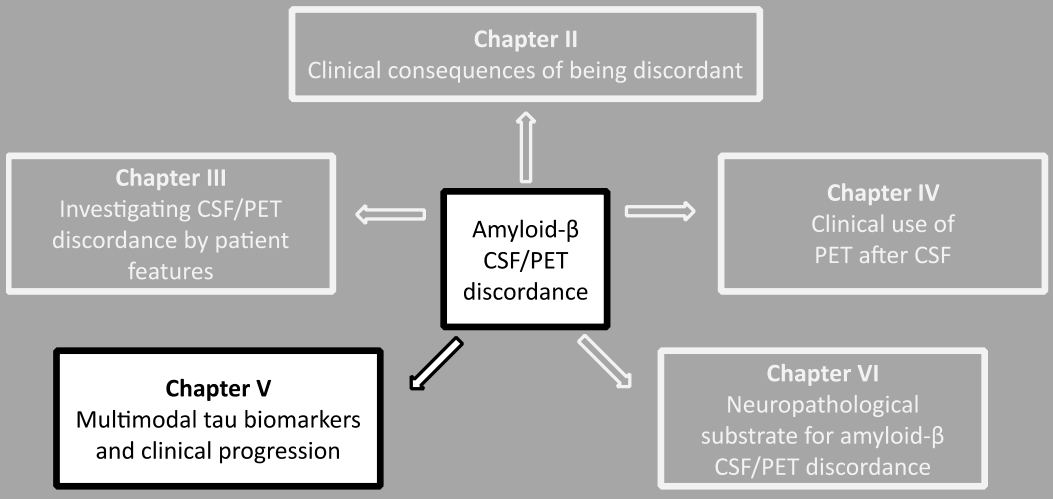
Chapter II
Clinical consequences of being discordant
Chapter III
Investigating CSF/PET discordance by patient features
Amyloid-β CSF/PET discordance
Chapter IV
Clinical use of PET after CSF
Chapter V
Multimodal tau biomarkers and clinical progression
Chapter VI
Neuropathological substrate for amyloid-β CSF/PET discordance

CHAPTER V: Association of amyloid-β CSF/PET discordance and tau load five years later

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ABSTRACT

OBJECTIVE: To investigate the association between discordant β-amyloid (Aβ) PET and CSF biomarkers at baseline and the emergence of tau pathology 5 years later.

METHODS: We included 730 ADNI participants without dementia (282 cognitively normal, 448 mild cognitive impairment) with baseline [^{18}F]Florbetapir PET and CSF $A\beta_{42}$ available. Aβ CSF/PET status was determined at baseline using established cut-offs. Longitudinal data was available for [^{18}F]florbetapir (Aβ) PET (baseline to 4.3±1.9 years), CSF (p)tau (baseline to 2.0±0.1 years), cognition (baseline to 4.3±2.0 years), and [^{18}F]flortaucipir (tau) PET (measured 5.2±1.2 years after baseline to 1.6±0.7 years later). We used linear mixed modelling to study the association between Aβ CSF/PET status and tau pathology measured in CSF or using PET. Additionally, we calculated the proportion of CSF+/PET- participants who during follow-up (1) progressed to Aβ CSF+/PET+ or (2) became tau-positive based on [^{18}F]flortaucipir PET.

RESULTS: Aβ CSF+/PET+ (n=318) participants had elevated CSF (p)tau levels and worse cognitive performance at baseline, while CSF+/PET- (n=80) participants were overall similar to the CSF-/PET- (n=306) group. Five years after baseline, [^{18}F]flortaucipir PET uptake in the CSF+/PET- group (1.20±0.13) did not differ from CSF-/PET- (1.18±0.08, $p=0.69$), but was substantially lower than CSF+/PET+ (1.48±0.44, $p<0.001$). Of the CSF+/PET- subjects, 21/64 (33%) progressed to Aβ CSF+/PET+, whereas only one (3%, difference $p<0.05$) became tau-positive based on [^{18}F]flortaucipir PET.

CONCLUSIONS: Aβ load detectable by both CSF and PET seems to precede substantial tau deposition. Compared to participants with abnormal Aβ levels on both PET and CSF, the CSF+/PET- group has a distinctly better prognosis.

INTRODUCTION

β-amyloid (Aβ) plaques and neurofibrillary tau tangles are considered the pathological hallmarks of Alzheimer's disease (AD).[1] Aβ pathology can be measured *in vivo* directly by quantifying the fibrillary depositions using PET, or indirectly by detecting the decrease of soluble $A\beta_{42}$ in CSF. Although these 2 measures are sometimes considered interchangeable,[2–4] 10%-20% cases show discordant results, especially at earlier stages of AD.[5–7] Therefore, it has been proposed that decreased $A\beta_{42}$ in CSF without significant tracer uptake on PET (i.e. Aβ CSF+/PET-) marks the pathological beginnings of Aβ accumulation.[8,9] This provides a powerful model to study the dynamic changes in Aβ as well as tau pathology during the earliest stages in the disease course of AD. Although CSF tau biomarkers have been available for over 2 decades,[10] tau PET using radiotracers such as [^{18}F]flortaucipir[11,12] has only more recently been developed. Tau PET offers the unique opportunity to study the spatial distribution of tau aggregates *in vivo*.

An open question to date is whether isolated Aβ positivity in CSF is followed by significant tau deposition already at this stage, or whether it will be subsequent to more advanced Aβ pathology detectable by both modalities (i.e. CSF and PET). As tau has a stronger correlation to neurodegeneration and cognitive function than Aβ accumulation,[13,14] this is of high clinical relevance. Furthermore, a better understanding of the interplay between the AD hallmark pathologies in early disease stages is crucial for the timing of interventions, as emerging treatments will likely be most effective when substantial neurodegeneration has not yet developed.[15] The aim of this study was therefore to use multimodal tau biomarkers and cognitive tests to explore the Aβ CSF/PET discordance.

METHODS

Participants

Data for this study was downloaded from the Alzheimer's Disease Neuroimaging Initiative (ADNI) website (http://adni.loni.usc.edu/), which also includes information about ADNI inclusion criteria and the procedure of biomarker acquisition.[16] We selected all ADNI participants, who had at least 1 [^{18}F]florbetapir Aβ PET scan and a CSF $A\beta_{42}$ analysis available within 1 year. The diagnosis closest to baseline [^{18}F]florbetapir PET within 1 year was used as the baseline diagnosis. In total, we included 730 subjects without dementia, of whom 282 were cognitively normal (CN) and 448 had mild cognitive impairment (MCI) at baseline.

We used Mini-Mental State Examination (MMSE) to assess global cognition and composite z-scores to assess memory[17] and executive functioning.[18] Longitudinal cognitive assessment was available for 711 (97%) subjects, with a median follow-up time of 4.2 (interquartile range [IQR] 2.9, 5.9) years. Similarly, a follow-up diagnosis was available for 724 (99%) participants with a median interval between baseline and follow-up of 4.1 (IQR 2.2, 5.9) years.

Standard protocol approvals, registrations, and patient consents

Written informed consent was obtained for all participants, and study procedures were approved by the institutional re- view board at each of the participating centers. ADNI is listed in the ClinicalTrials.gov registry (ADNI-1: NCT00106899; ADNI-GO: NCT01078636; ADNI-2: NCT0123197).

[^{18}F]florbetapir PET

Acquisition and processing of [^{18}F]Florbetapir PET using Freesurfer was performed as described previously.[19,20] At least one follow-up PET scan was available for 579 (79%) of participants with a median follow-up time of 4.1 (IQR 2.1, 5.9) years. We used a neocortical composite score provided by ADNI consisting of the mean uptake in the frontal, cingulate, parietal and temporal regions. We created standardized uptake value ratios (SUVRs) using whole cerebellum as the reference region and used an SUVR cut-off of 1.11 to determine binarized Aβ status based on PET.[21,22] For longitudinal linear mixed modelling, we additionally used SUVR values in 34 regions from the Desikan-Killiany atlas using (i) the whole cerebellum[23] and (ii) a composite white matter region as reference regions, because the latter has been shown to be more reliable in longitudinal analyses.[24] Finally, we used an early composite region identified in a recent study[25] (including bilateral precuneus, posterior cingulate, insula, and medial and lateral orbitofrontal regions) to capture the early accumulation of Aβ .

CSF studies

Lumbar punctures were performed as previously described.[26] CSF samples were frozen on dry ice after collection and transported to the UPenn Medical Center ADNI Biomarker Core laboratory. Thereafter, 0.5mL aliquots were prepared and stored in polypropylene tubes at -80°C. CSF samples were analyzed for $A\beta_{42}$, total tau (t-tau) and phosphorylated tau (p-tau) using the AlzBio3 assays (Fujirebio) on the xMAP platform (Luminex). In case samples were reanalyzed, we used the median value of those results. A cutoff of 192 pg/mL was used to determine Aβ status based on

CSF.[26,27] Longitudinal CSF analyses were available for 297 (41%) of participants with the median follow-up time of 2.0 (IQR 2.0, 2.0) years.

[^{18}F]flortaucipir PET

[^{18}F]Flortaucipir PET was performed at each ADNI site according to standardized protocols. Images were acquired via 4 x 5 minute frames from 80 to 100 minutes after injecting ~370 MBq of [^{18}F]flortaucipir. [^{18}F]flortaucipir PET was available for 253 (35%) participants and was performed a median of 5.2 (IQR 4.2, 6.1) years after baseline [^{18}F]florbetapir PET, allowing measuring tau pathology at a significantly later time-point. Of these participants, 110 had 1 follow-up scan after 1.3 (IQR 1.0, 2.1) years. We used Freesurfer-defined Desikan-Killiany atlas regions provided by ADNI that were created by co-registering the [^{18}F]flortaucipir image with a previously parcellated and segmented magnetic resonance imaging MPRAGE from the same time.[28] Thereafter, we created 3 bilaterally volume-weighted composite regions to cover the full spectrum of tau aggregation: entorhinal cortex, temporal meta-ROI reflecting Braak stage I to IV (including entorhinal, parahippocampal cortex, amygdala, fusiform, inferior and middle temporal cortices), and Braak stage V and VI (including wider neocortical areas).[29–31] Cut-offs (1.39, 1.34, and 1.28 SUVR, respectively) obtained using a similar PET pipeline were used to determine [^{18}F]flortaucipir positivity.[32]

Statistical analysis

We selected the first available [^{18}F]florbetapir PET as baseline Aβ PET, and CSF Aβ_{42} closest in time within 1 year to the [^{18}F]florbetapir PET as baseline CSF. Thereafter, we created four groups based on the binarized Aβ status on PET and CSF: concordantly amyloid-negative (CSF-/PET-), concordantly amyloid-positive (CSF+/PET+), discordantly amyloid-positive based on CSF (CSF+/PET-) or PET (CSF-/PET+). Participant groups were compared using χ^2 tests, 2 sample t-tests and Wilcoxon rank-sum tests. Statistical analysis was performed using R software version 3.6.3.[33–36]

We used linear mixed modelling to investigate longitudinal changes for (1) regional Aβ burden assessed by [^{18}F]florbetapir PET, (2) tau pathology assessed by CSF t-tau, p-tau (measured from baseline) and [^{18}F]flortaucipir PET (first measured 5 years after baseline), and (3) cognitive measures (MMSE, and ADNI memory and executive composite scores). The models included time in years as a continuous variable, Aβ CSF/PET group, and an interaction between time*CSF/PET group. All models also included terms for age and sex. The models predicting regional Aβ PET and tau pathology based on CSF or PET were additionally adjusted for MMSE to account for clinical disease severity. The models predicting cognitive test results were additionally

adjusted for education. We used a random intercept and a random slope for all models. We first selected CSF-/PET- as the reference group and interpreted the main effect of CSF/PET group status (CSF-/PET+ and CSF+/PET-) in the models as difference at baseline, and the CSF/PET group*time interaction term as the change over time. Thereafter, we repeated this analysis with CSF+/PET+ as the reference group. We also tested whether these effects are consistent (1) when using random samples from the CSF+/PET+ group in order to achieve a similar 50%/50% CN/MCI ratio compared the other groups, or (2) when covarying for *APOE* ε4 carriership. We then performed Kaplan-Meier survival analyses to investigate the association between Aβ CSF/PET status and clinical progression for CN participants (progression to MCI or dementia) and participants with MCI (progression to dementia). We additionally conducted Cox regression analyses to obtain *post hoc* Hazard Ratios (HRs) for each of the Aβ CSF/PET profiles.

Finally, we performed two analyses in CSF+/PET- participants only. First, to investigate subthreshold levels of tau pathology in the Aβ CSF+/PET- group, we performed linear regression models with Aβ pathology measured by [^{18}F]florbetapir PET (globally as well as in early accumulating regions[25]) at baseline as the predictor, with cross-sectional tau pathology measured by either CSF t-tau or p-tau (at baseline) or the 3 composite regions of [^{18}F]flortaucipir PET (5 years later) as the outcome. Second, we investigated our hypothesis that progression from Aβ CSF+/PET- to CSF+/PET+ occurs at a higher rate than progression to tau positivity based on [^{18}F]flortaucipir PET. We calculated the proportions of CSF+/PET- participants, who (1) during the follow-up period converted into the CSF+/PET+ group based on the last available [^{18}F]florbetapir PET scan and CSF analysis, and (2) whose last available [^{18}F]flortaucipir PET was positive in any of the 3 composite regions. We compared these outcomes by testing for overlapping 95% confidence intervals on estimated proportions. Last available [^{18}F]flortaucipir PET was performed a median of 6.0 [IQR: 5.5, 6.8] years after baseline and 0.0 [-2.0, 1.4] years from last available [^{18}F]florbetapir PET.

Data availability

All imaging, demographics, and neuropsychological data used in this article are publicly available and were downloaded from the ADNI website (www.adni.loni.usc.edu). Upon request, we will provide a list of ADNI participant identifications for replication purposes.

RESULTS

Study participants

Of the study participants, 306 (42%) were CSF-/PET-, 80 (11%) CSF+/PET-, 26 (4%) CSF-/PET+ and 318 (44%) CSF+/PET+. Characteristics were overall similar between CSF-/PET- and the two discordant groups. Participants in the CSF+/PET+ group were older at baseline and at symptom onset, had a higher proportion of *APOE* ε4 carriers, were more often diagnosed with MCI, had lower cognitive scores, higher CSF (p)tau levels at baseline and higher [^{18}F]flortaucipir PET uptake five years later (**Table 1**; also data available from Dryad, **Table 2**, https://doi.org/10.5061/dryad.c59zw3r49).

Table 1. Study participants

	CSF-/PET-	CSF+/PET-	CSF-/PET+	CSF+/PET+
N (%)	306 (42)	80 (11)	26 (4)	318 (44)
Male (%)	161 (53)[c]	48 (60)[c]	6 (23)[abd]	165 (52)[c]
Age, y	72 (7)[d]	72 (8)	71 (6)[d]	74 (7)[ac]
Education, y	17 (14, 18)	16 (16, 18)	16 (14, 18)	16 (14, 18)
APOE ε4 carriership	53 (17)[bd]	32 (40)[ad]	8 (31)[d]	203 (64)[abc]
Diagnosis, MCI	155 (51)[d]	41 (51)[d]	13 (50)[d]	239 (75)[abc]
[^{18}F]florbetapir PET composite SUVR	1.01 (0.98, 1.04)[bcd]	1.05 (1.00, 1.08)[acd]	1.15 (1.13, 1.19)[abd]	1.36 (1.26, 1.49)[abc]
CSF $A\beta_{42}$	232 (213, 249)[bcd]	164 (149, 181)[acd]	214 (204, 243)[abd]	136 (121, 154)[abc]
Cognitive test scores (baseline):				
MMSE	28.8 (1.4)[d]	28.8 (1.4)[d]	28.6 (1.6)	28 (1.8)[ab]
ADNI memory composite	0.90 (0.68)[d]	0.76 (0.64)[d]	1.09 (0.48)[d]	0.30 (0.71)[abc]
ADNI executive composite	0.85 (0.85)[bd]	0.55 (0.74)[acd]	1.13 (0.77)[bd]	0.27 (0.86)[abc]
CSF tau measures (baseline):				
CSF t-tau	53 (42, 68)[d]	55 (39, 76)[d]	57 (48, 74)[d]	93 (68, 135)[abc]
CSF p-tau	25 (20, 35)[d]	24 (19, 37)[d]	26 (21, 43)[d]	47 (35, 65)[abc]
[^{18}F]flortaucipir PET (5 years later):				
Entorhinal SUVR	1.12 (1.06, 1.17)[d]	1.13 (1.08, 1.19)[d]	1.11 (1.06, 1.15)[d]	1.40 (1.21, 1.61)[abc]
Temporal meta-ROI SUVR	1.18 (1.13, 1.23)[d]	1.18 (1.15, 1.23)[d]	1.18 (1.16, 1.24)[d]	1.36 (1.22, 1.58)[abc]
BRAAK V and VI SUVR	1.06 (1.02, 1.10)[cd]	1.08 (1.01,1.10)[d]	1.11 (1.07,1.17)[a]	1.16 (1.06,1.25)[ab]

Abbreviations: ADNI = Alzheimer's Disease Neuroimaging Initiative; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; p-tau = phosphorylated tau; ROI = region of interest; SUVR = standardized uptake value ratio; t-tau = total tau. Values are n (%), mean (SD), or median (interquartile range).

Accumulation of Aβ

First, we assessed regional [^{18}F]florbetapir patterns across groups (**Figure 1**; also data available from Dryad, **Table 3**, https://doi.org/10.5061/dryad.c59zw3r49). Although the CSF-/PET+ group had more tracer uptake at baseline than CSF-/PET-, they did not accumulate significantly more Aβ over time on PET. Over time, CSF+/PET- group had widespread increase of tracer uptake compared to CSF-/PET- irrespective of the reference region. Additionally, CSF+/PET- had slightly more tracer uptake at baseline than CSF-/PET-. These findings were consistent when covarying for *APOE* ε4 status (data available from Dryad, **Figure 5**, https://doi.org/10.5061/dryad.c59zw3r49).

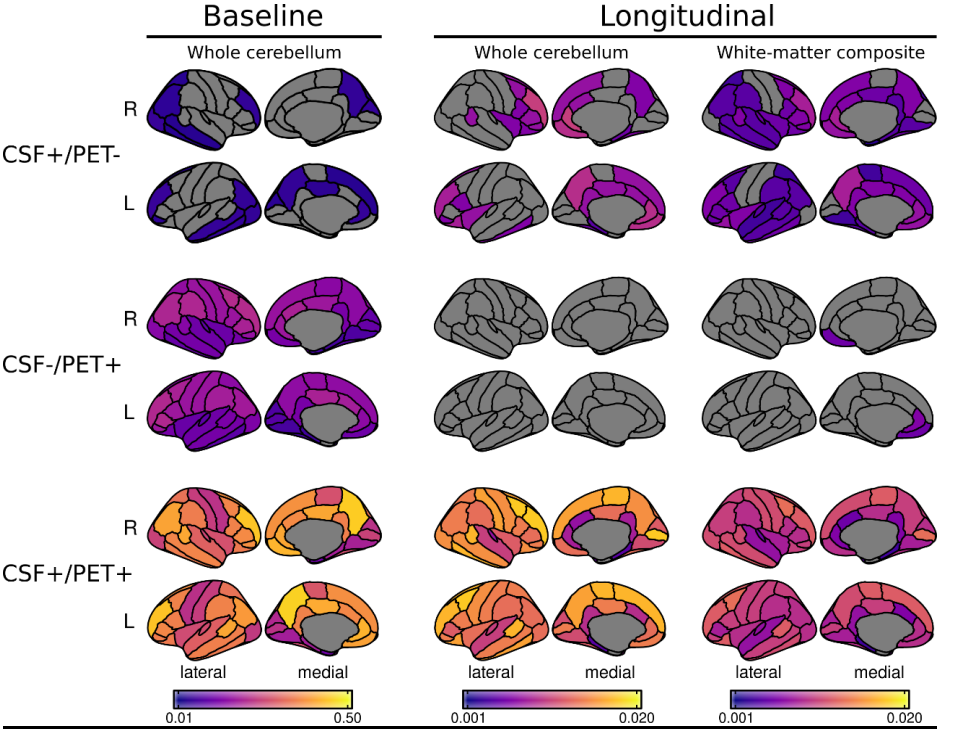

Figure 1. Accumulation of β-amyloid measured by [^{18}F]florbetapir positron emission tomography

Results obtained from linear mixed models, with the colors indicating β-coefficients relative to the CSF-/PET- group. The 3 sections show the difference between the group of interest (CSF+/PET-, CSF-/PET+ or CSF+/PET+) and CSF-/PET-. The first column shows the β-coefficient for the baseline effect of the CSF/PET group when using whole cerebellum as the reference region. The second and third columns show the β-coefficients for the interaction between CSF/PET group and time as the longitudinal change when using whole cerebellum or composite white matter as the reference, respectively. Only regions with $p<0.05$ are shown. Image was created using the ggseg package in R.

Longitudinal trajectories of tau and cognition

Next, we investigated longitudinal trajectories of tau pathology (CSF and PET) and cognition (**Figure 2**; also data available from Dryad, **Table 4**, https://doi.org/10.5061/dryad.c59zw3r49). Compared to Aβ CSF+/PET+ group, participants with discordant CSF/PET Aβ status had at baseline significantly lower levels of CSF t-tau and p-tau measures (both p<0.001) and better cognitive test scores (MMSE: p=0.001 for CSF+/PET-, p=0.201 for CSF-/PET+; cognitive test scores (MMSE: p=0.001 for CSF+/PET-, p=0.201 for CSF-/PET+; memory: both p<0.001; executive functioning: p=0.010 for CSF+/PET-, p<0.001 for CSF-/PET+). Longitudinally, participants in both CSF/PET groups showed slower decline in cognitive test scores (all p<0.001 for both), compared to the CSF+/PET+ group. [^{18}F]flortaucipir PET was first performed 5 years after baseline. Participants in both discordant CSF/PET groups had less [^{18}F]flortaucipir uptake in entorhinal cortex (both p<0.001), temporal meta-ROI (p<0.001 for CSF+/PET-, p=0.006 for CSF-/PET+) and Braak V/VI (p=0.002 for CSF+/PET-, p=0.274 for CSF-/PET+) compared to CSF+/PET+. Longitudinally, discordant CSF/PET groups had lower rates of increase in [^{18}F]flortaucipir uptake in temporal meta-ROI (p=0.010 for CSF+/PET-, p=0.031 for CSF-/PET+) and Braak V/VI composite areas (p=0.022 for CSF+/PET-). CSF+/PET- participants additionally had at baseline worse executive functioning than participants in the CSF-/PET- group (p=0.034). group. The CSF/PET discordant groups were otherwise similar to CSF-/PET-. These findings were consistent when drawing random samples from the CSF+/PET+ group in order to achieve a 50/50 CN/MCI ratio in CSF+/PET+ group and when covarying for *APOE* ε4 status (data available from Dryad, **Table 5** and **6**, respectively, https://doi.org/10.5061/dryad.c59zw3r49).

Clinical Progression

Next, we investigated the association between Aβ CSF/PET status and clinical progression using Kaplan-Meier estimates and Cox regression analyses (**Figure 3**; also data available from Dryad, **Table 7**, https://doi.org/10.5061/dryad.c59zw3r49). Progression from MCI to dementia occurred less often in Aβ CSF+/PET- (7%, hazard ratio [HR]= 0.10 [0.03, 0.32], p<0.001) and CSF-/PET- (8%, HR= 0.11 [0.06, 0.20], p<0.001) participants, compared to the CSF+/PET+ (48%) group. Similarly, in CN participants, clinical progression to MCI or dementia occurred most often in CSF+/PET+ (30%), compared to CSF-/PET- (12%, HR= 0.33 [0.18, 0.62], p<0.001), CSF-/PET+ (15%, HR= 0.42 [0.10, 1.77], p=0.236), CSF+/PET- (21%, HR= 0.55 [0.24, 1.22], p=0.141). No participants with Aβ CSF-/PET+ progressed to dementia.

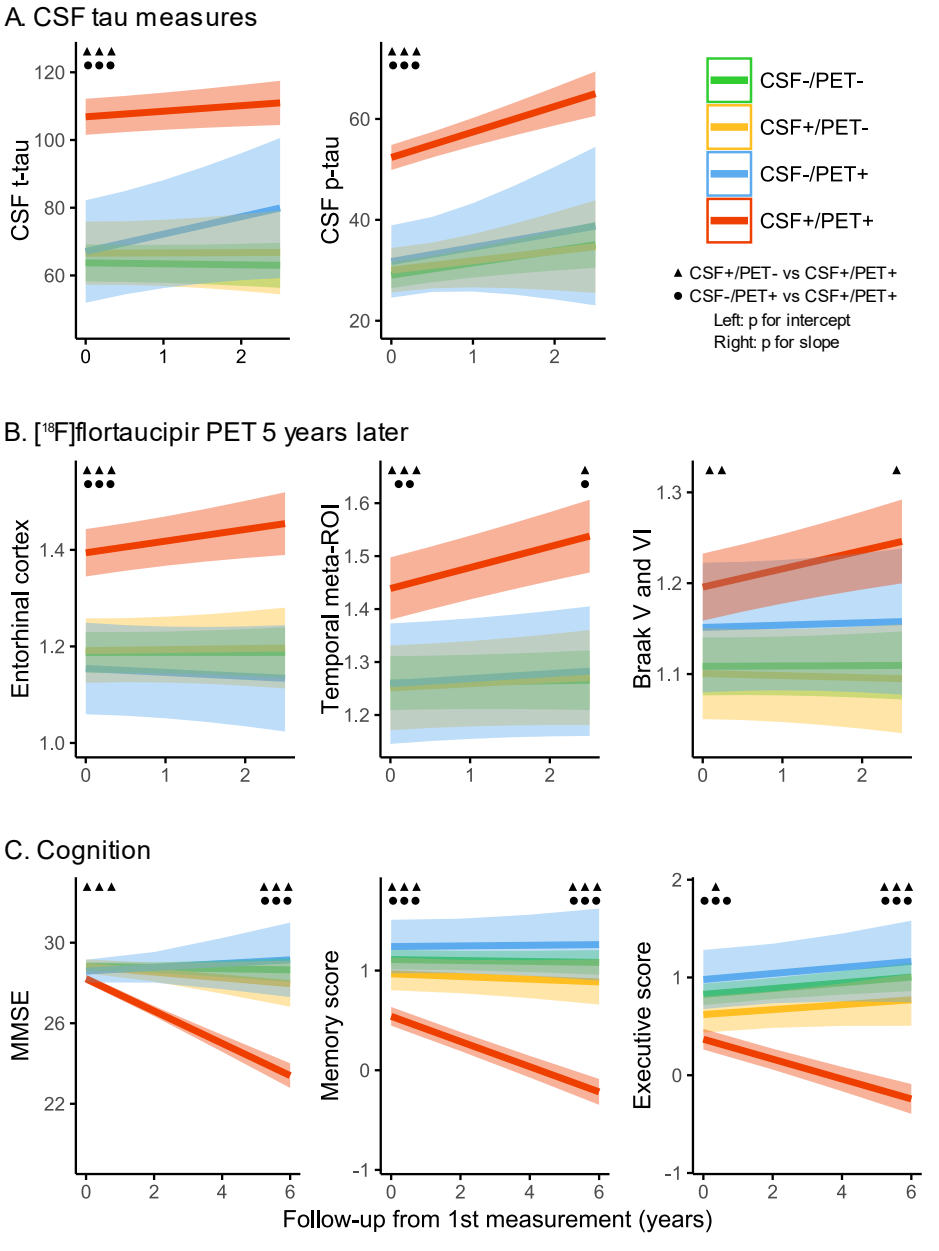

Figure 2. Longitudinal trajectories of tau pathology and cognition

Results obtained from linear mixed models investigating the effect of amyloid-β CSF/PET discordance on (A) tau pathology assessed by CSF t-tau and p-tau, (B) tau pathology based on regional [^{18}F]flortaucipir PET, and (C) cognitive trajectories measured by Mini-Metal State Examination (MMSE), Alzheimer's Disease Neuroimaging Initiative memory and executive composite scores. CSF tau was assessed from baseline to median 2.0 years; [^{18}F]flortaucipir PET (Continued from previous page) was first performed a median of 5.2 years after baseline and was followed up a median of 1.3 years later; cognitive tests were assessed at

(Continued from previous page) baseline, and followed up for a median of 4.2 years. Difference from CSF+/PET+ is illustrated by black triangles (for CSF+/PET-) or circles (for CSF-/PET+) with the number of symbols indicating statistical difference (1: p<0.05; 2: p<0.01; 3: p<0.001). Symbols on the left side of a plot show difference in intercept (main effect of CSF/PET group in the model), and symbols on the right side show a difference in slope (interaction between CSF/PET group and time). CSF+/PET- participants also had at baseline worse executive functioning than participants in the CSF-/PET- group (p=0.034). Image was created using the ggeffects package in R. ROI = region of interest.

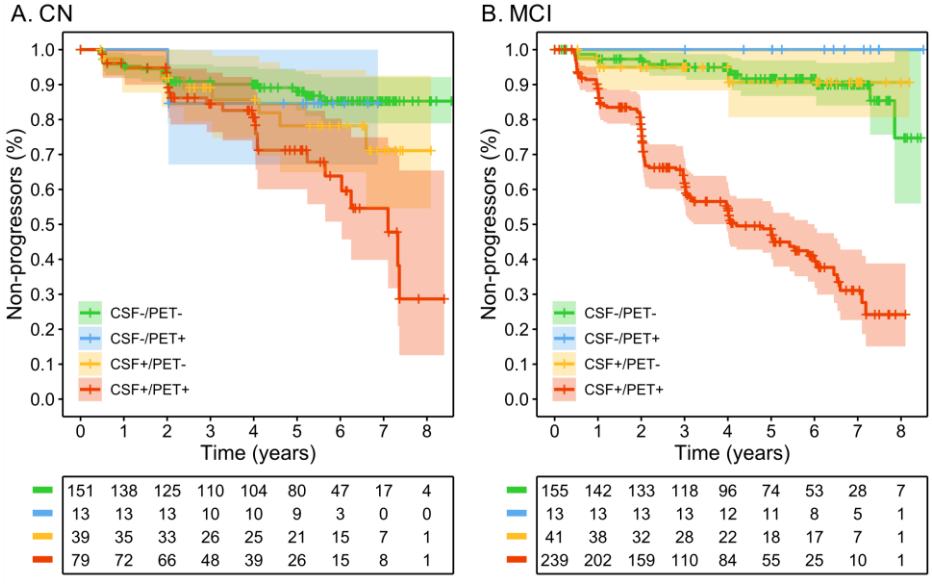

Figure 3. Kaplan-Meier curves for clinical progression

Results obtained from the Kaplan-Meier estimate investigating the association between β-amyloid CSF/PET profile and clinical progression for cognitively normal (CN) participants (progression to MCI or dementia; A) or participants with MCI (progression to dementia; B). Tables below the figures report per year the number of participants with available follow-up data.

Replication involving only participants with available [^{18}F]flortaucipir PET

We repeated the previous analyses involving only the 253 participants with available [^{18}F]flortaucipir PET (data available from Dryad, Table 8-10, https://doi.org/10.5061/dryad.c59zw3r49). The effects of Aβ CSF/PET groups on regional [^{18}F]florbetapir uptake (**Figure 6** and **Table 11** from Dryad), trajectories of CSF tau and cognition (**Figure 7** and **Table 12** from Dryad) and clinical progression (**Figure 8** and **Table 13** from Dryad) were consistent with the aforementioned findings in the full sample.

Associations between Aβ and tau in CSF+/PET- participants

We then assessed whether continuous [^{18}F]florbetapir uptake in the subthreshold range is correlated with tau measures in the Aβ CSF+/PET- group (**Figure 4**). [^{18}F]florbetapir tracer uptake globally and in early accumulating regions was associated with higher baseline CSF t-tau (p=0.001 and p=0.003), and higher [^{18}F]flortaucipir uptake in entorhinal cortex (p=0.003 and p=0.010) and marginally in the temporal meta-ROI (p=0.062 and p=0.091).

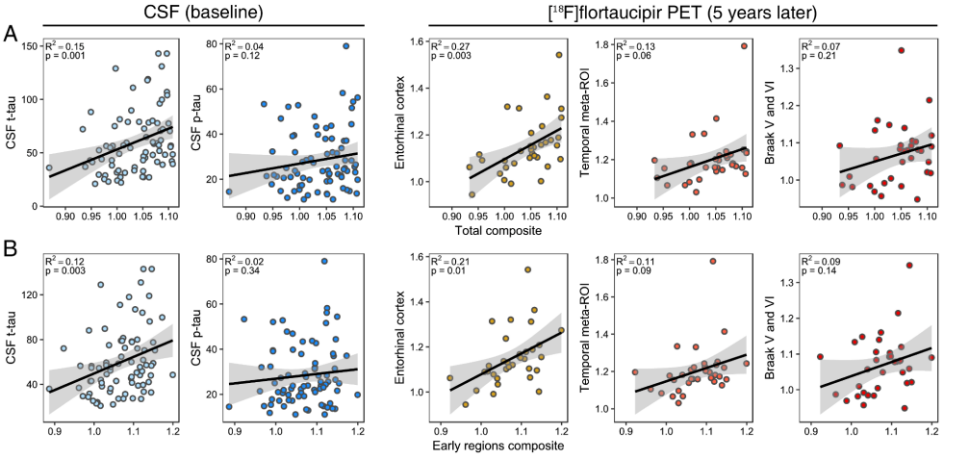

Figure 4. Correlation between baseline [^{18}F]florbetapir PET and tau pathology based on CSF and PET.

Plotted is the association between [^{18}F]florbetapir PET, based on (A) total composite and (B) early accumulating regions composite on the x axis, and cross-sectional tau pathology, measured by either CSF t-tau or p-tau at baseline or [^{18}F]flortaucipir PET median of 5.2 years after baseline on the y axis. R^2 and p values are reported from the from linear regression models, which were also adjusted for the times between baseline [^{18}F]florbetapir PET and the outcome modality.

Accumulation of Aβ and tau in CSF+/PET- participants

Finally, we investigated whether CSF+/PET- participants progress to CSF+/PET+ or become tau-positive first. Based on the last available [^{18}F]florbetapir Aβ PET scan and CSF $Aβ_{42}$ analysis, 21/64 (33%) of the baseline Aβ CSF+/PET- participants progressed to CSF+/PET+. Of 34 CSF+/PET- participants with [^{18}F]flortaucipir PET available, 11/34 (32% [95% confidence interval: 17%, 48%]) progressed to CSF+/PET+, but only 1 (3% [95% confidence interval: -3%, 9%], difference in proportions p<0.05) turned tau-positive based on entorhinal or temporal meta-ROI regions, and none in Braak V/VI. For comparison, 2/123 (2%, p=1.00) of Aβ CSF-/PET-, 0/16 (0%, p=1.00) CSF-/PET+,

and 47/80 CSF+/PET+ (59%, p<0.001) were tau-positive based on last available [^{18}F]flortaucipir PET.

DISCUSSION

We investigated the association between discordant CSF/PET Aβ biomarkers on tau pathology and clinical progression. Our main finding was that although Aβ CSF+/PET- participants showed longitudinal accumulation of Aβ based on PET, they had significantly less tau pathology based on both CSF at baseline and [^{18}F]flortaucipir 5 years later compared to participants with CSF+/PET+ Aβ status. Similarly, discordant Aβ status was associated with better cognitive outcome and a lower risk of clinical progression than CSF+/PET+. We also showed that during follow-up, CSF+/PET- subjects frequently progressed to Aβ CSF+/PET+, whereas only one participant reached the threshold of tau-positivity based on [^{18}F]flortaucipir PET. Finally, we showed a correlation between tau measures and global Aβ PET tracer uptake, indicating possible subthreshold accumulation of AD pathology in CSF+/PET- subjects. Taken together, our findings suggest that CSF+/PET- Aβ status is associated with a distinctly better prognosis than CSF+/PET+, and that a sufficient Aβ load detectable by both CSF and PET seems to precede significant tau deposition.

Using both CSF tau measures and [^{18}F]flortaucipir PET we found that participants with discordant CSF/PET Aβ status had less tau pathology than CSF+/PET+ Aβ participants, and comparable tau load as observed in concordant Aβ negative participants. It has been proposed that CSF+/PET- status can be caused by CSF Aβ$_{42}$ being able to detect Aβ at an earlier stage due to the decrease of soluble Aβ$_{42}$ in CSF preceding fibrillary depositions visualized by PET. This is supported by higher rates of CSF+/PET- compared to CSF-/PET+ across several studies.[7,37,38] Previous longitudinal PET studies have shown that subjects with CSF+/PET- Aβ status show significant accumulation of Aβ over time.[8,9,39] We replicated this finding in our study, further supporting the notion that CSF+/PET- Aβ status identifies the beginnings of Aβ accumulation. Although participants with CSF-/PET+ Aβ status had higher [^{18}F]florbetapir tracer uptake at baseline, they did not show significant accumulation of Aβ over time. Combined with the lack of clinical progression in this group, these observations suggest that isolated Aβ PET positivity might be caused by non-specific tracer uptake in the white matter, processing errors or other unknown factors.[6] In CSF+/PET- participants, the lack of substantial tau pathology based on CSF tau measures at baseline and on [^{18}F]flortaucipir PET five years later, accompanied by lack of cognitive decline and clinical progression, suggests these subjects have a distinctly more favorable prognosis than subjects with CSF+/PET+. This is likely caused by the remarkably slow course of AD, which is characterized by gradual accumulation of

pathology over time.[40,41] Accounting for the more benign prognosis of CSF+/PET- subjects is important for the timing of future interventions at the earliest stages of AD pathology.

Current hypothetical biomarker models suggest that accumulation of Aβ pathology is followed by detectable cortical tau pathology, subsequently leading to neurodegeneration and cognitive decline.[42,43] Although it has been proposed that CSF+/PET- is followed by conversion to CSF+/PET+,[8,44] the exact timing of CSF+/PET- status in regard to that timeline, in particular towards accumulation of tau, is unknown. We found that within 5 years, one-third of the CSF+/PET- participants progressed to CSF+/PET+, whereas at that time only one participant exhibited suprathreshold early to intermediate stage tau pathology based on [^{18}F]flortaucipir PET and none showed widespread neocortical uptake (i.e. Braak stage V/VI regions). This finding has at least 2 implications. First, as the majority of CSF+/PET- participants did not progress to CSF+/PET+ within 5 years, this indicates that in the majority of cases the CSF+/PET- Aβ status lasts for several years. Second, accumulation of sufficient Aβ detectable by both CSF and PET seems to precede significant accumulation of tau pathology.[4546] However, we also found a correlation between baseline regional Aβ PET and tau pathology in the CSF+/PET- group, suggesting that there already might be interaction present between Aβ and tau. This supports previous work, emphasizing the importance of considering subthreshold accumulation of pathology to better understand disease mechanisms of early preclinical stages of AD.[47–49]

Our study has some limitations. Although ADNI is one of the largest cohorts with both available Aβ PET and CSF analysis, only a relatively small number of participants with discordant CSF/PET Aβ status were available. Second, our main outcome measures of tau pathology based on CSF and PET were assessed at different time-points. Although that reduces the direct comparability of these findings, they also complement each other and allow to measure tau pathology both at baseline and several years later. Relatively short follow-up periods were available for both CSF tau measures and [^{18}F]flortaucipir PET. Therefore, it is possible, that with longer follow-up periods, subjects with discordant Aβ status might show diverging trajectories compared to the CSF-/PET- group. Our interpretation of the study could also be affected by the possibility that CSF+/PET- status might reflect a different subtype of AD, although no evidence for that exists. Finally, cut-offs of biomarkers as well as defining Aβ PET status based on global SUVR are important considerations when evaluating these results. As suboptimal cut-offs might result in misclassification,[50] we used applied widely used and validated cut-offs for both PET and CSF.

Our findings indicate that a sufficient Aβ load detectable by both PET and CSF seems to precede substantial tau deposition. Subjects with CSF+/PET- Aβ profile are at a

significantly earlier clinical and biological disease stage than those with CSF+/PET+, and have a distinctly better prognosis for at least 5 years.

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Author contributions: J Reimand - Study design; analysis and interpretation of data; writing and revision of the manuscript; and statistical analysis. L Collij - Acquisition and analysis of data; and critical revision the manuscript for intellectual content. P Scheltens - Acquisition and analysis of data; and critical revision the manuscript for intellectual content. F Bouwman - Acquisition and analysis of data; and critical revision the manuscript for intellectual content. R Ossenkoppele - Study concept and design; interpretation of data; critical revision of the manuscript for intellectual content; and supervision of the study

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Glossary: Aβ – β-amyloid, AD – Alzheimer's disease, ADNI – Alzheimer's disease neuroimaging initiative, CSF – Cerebrospinal fluid, IQR – Interquartile range, MCI – Mild cognitive impairment, MMSE – Mini mental state examination, MRI – Magnetic resonance imaging, PET – Positron emission tomography, p-tau – phosphorylated tau, ROI – Region of interest, SUVR – Standardized uptake value ratio, t-tau – total tau.

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SUPPLEMENTARY DATA

Table 2. Time of symptom onset by CSF/PET groups

A. CN (n =282):

	CSF-/ PET-	CSF+/ PET-	CSF-/ PET+	CSF+/ PET+	p
n	151	39	13	79	
Age at cognitive symptoms onset, years (mean (SD))	68 (3)	70 (9)	62 (NA)	69 (6)	NA
Time between cognitive symptoms onset and baseline amyloid-β PET, years (mean (SD))	5 (3)	3 (1)	5 (NA)	4 (3)	NA
Available for participants, n (%):	26 (17%)	4 (10%)	1 (8%)	10 (13%)	

B. MCI (n = 448):

	CSF-/ PET-	CSF+/ PET-	CSF-/ PET+	CSF+/ PET+	p
n	155	41	13	239	
Age at cognitive symptoms onset, years (mean (SD))	65 (8)^{D}	66 (9)	63 (12)	69 (7)^{A}	0.0016
Time between cognitive symptoms onset and baseline amyloid-β PET, years (mean (SD))	5 (3)	5 (3)	6 (8)	4 (3)	0.1793
Available for participants, n (%):	93 (60%)	30 (73%)	7 (53%)	175 (73%)	
Age at MCI symptoms onset, years (mean (SD))	66 (9)^{D}	62 (8)^{D}	66 (3)	71 (7)^{AB}	0.0038
Time between MCI symptoms onset and baseline amyloid-β PET, years (mean (SD))	4 (3)	4.73 (2)	3 (1)	4 (3)	0.4926
Available for participants, n (%):	51 (33%)	10 (24%)	6 (46%)	4(18%)	

Presented are the average age at cognitive symptom onset and the time between symptom onset and baseline amyloid PET per amyloid-β CSF/PET group for CN (A) and MCI (B) participants. Symptom onset years are estimations available in ANDI demographics file (variables PTCOGBEG, PTMCIBEG). A, B, D indicate differences ($p < 0.05$) from other groups: A – difference from CSF-/PET-; B – difference from CSF+/PET-; D – difference from CSF+/PET+. False discovery rate (FDR) correction was used to account for multiple comparison.

Table 3. Regional [18F]florbetapir uptake of CSF+/PET- vs CSF-/PET-

	Whole cerebellum reference				White matter composite reference			
	Intercept β	p	Slope β	p	Intercept β	p	Slope β	p
Total composite	0.031 (0.015)	0.03 3	0.006 (0.003)	0.02 0	0.034 (0.008)	<0.0 01	0.005 (0.001)	<0.0 01
Parietal	0.035 (0.015)	0.02 5	0.005 (0.003)	0.07 1	0.037 (0.010)	<0.0 01	0.004 (0.001)	0.002
Temporal	0.027 (0.014)	0.06 4	0.004 (0.002)	0.06 5	0.030 (0.009)	0.001	0.004 (0.001)	0.002
Cingulate	0.032 (0.017)	0.05 6	0.007 (0.003)	0.01 2	0.036 (0.009)	<0.0 01	0.006 (0.001)	<0.0 01
Frontal	0.032 (0.016)	0.04 3	0.007 (0.003)	0.00 7	0.035 (0.009)	<0.0 01	0.006 (0.001)	<0.0 01

Results obtained from linear mixed models showing regional [^{18}F]florbetapir uptake between CSF+/PET- and CSF-/PET- at baseline (intercept β) and longitudinally (slope β). Composite score standardized uptake values (SUV) were taken from the ANDI database and divided by the whole cerebellar or white matter composite region SUVs to create SUV ratios. The total composite score incorporates bilateral frontal (including caudal middle frontal, medial and lateral orbitofrontal, pars opercularis, pars orbitalis, pars triangularis, rostral middle frontal, superior frontal, frontal pole regions), cingulate (including caudal and rostral anterior, isthmus and posterior cingulate regions), lateral parietal (including inferior and superior parietal, supramarginal and precuneus regions), and temporal regions (including middle and superior temporal regions).

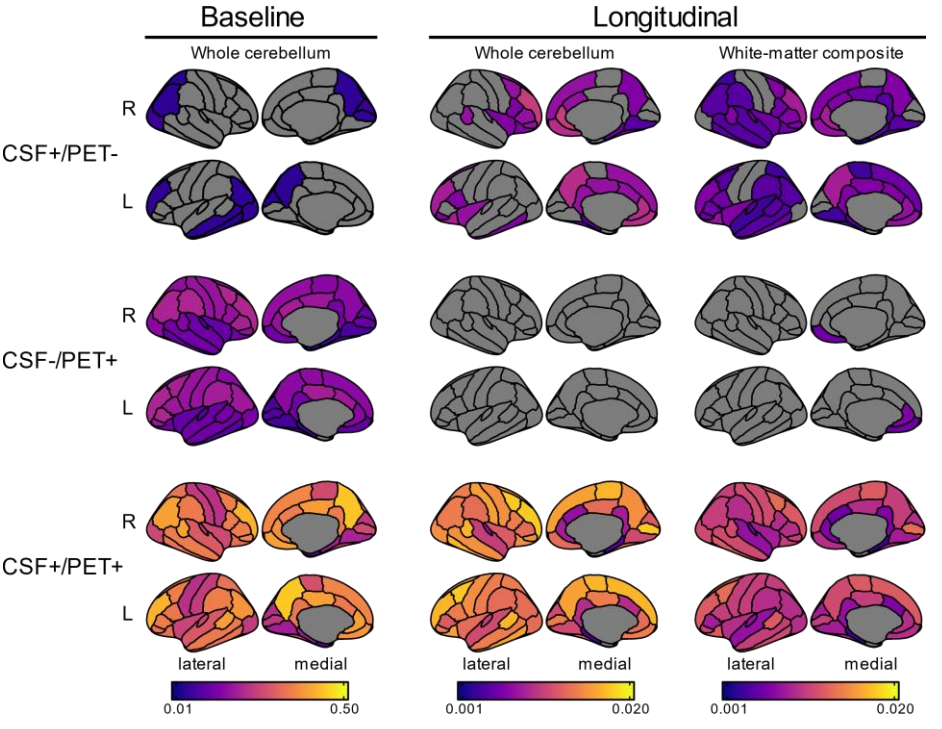


Figure 5. Accumulation of amyloid-β measured by [18F]florbetapir positron emission tomography covarying for APOE ε4 status

Results obtained from linear mixed models, with the colors indicating β-coefficients relative to the CSF-/PET- group. The three sections show the difference between the group of interest (CSF+/PET-, CSF-/PET+ or CSF+/PET+) and CSF-/PET-. The first column shows the β-coefficient for the baseline effect of the CSF/PET group when using whole cerebellum as the reference region. The second and third columns show the β-coefficients for the interaction between CSF/PET group and time as the longitudinal change when using whole cerebellum or composite white matter as the reference, respectively. Only regions with $p<0.05$ are shown. Image was created using the ggseg package in R.

Table 4. Longitudinal trajectories for tau pathology and cognition

			Intercept β (SD)	P difference from		Slope β (SD)	P difference from	
				CSF-/ PET-	CSF+/ PET+		CSF-/ PET-	CSF+/ PET+
CSF	t-tau	CSF-/PET-	164.7 (32.0)			-0.29 (0.87)		
		CSF-/PET+	168.1 (32.5)	0.677	**<0.001**	5.14 (3.07)	0.088	0.270
		CSF+/PET-	167.6 (32.3)	0.565	**<0.001**	0.10 (1.77)	0.843	0.423
		CSF+/PET+	207.9 (31.6)			1.65 (0.83)		
	p-tau	CSF-/PET-	78.3 (14.9)			2.42 (0.84)		
		CSF-/PET+	81.0 (15.2)	0.477	**<0.001**	2.81 (3.03)	0.901	0.472
		CSF+/PET-	79.3 (15.1)	0.670	**<0.001**	1.87 (1.74)	0.775	0.095
		CSF+/PET+	101.7 (14.7)			5.06 (0.79)		
[^{18}F]flor-taucipir PET	Entorhinal cortex	CSF-/PET-	2.27 (0.18)			0.00 (0.01)		
		CSF-/PET+	2.23 (0.18)	0.510	**<0.001**	-0.01 (0.01)	0.617	0.065
		CSF+/PET-	2.27 (0.18)	0.918	**<0.001**	0.00 (0.01)	0.875	0.149
		CSF+/PET+	2.47 (0.18)			0.02 (0.01)		
	Temporal meta-ROI	CSF-/PET-	2.98 (0.22)			0.00 (0.01)		
		CSF-/PET+	2.98 (0.22)	0.986	**0.006**	0.01 (0.01)	0.573	**0.031**
		CSF+/PET-	2.97 (0.22)	0.835	**<0.001**	0.01 (0.01)	0.615	**0.010**
		CSF+/PET+	3.16 (0.21)			0.04 (0.01)		
	Braak V and VI	CSF-/PET-	2.21 (0.14)			0.00 (0.00)		
		CSF-/PET+	2.25 (0.14)	0.260	0.274	0.00 (0.01)	0.832	0.113
		CSF+/PET-	2.20 (0.14)	0.770	**0.002**	0.00 (0.01)	0.765	**0.022**
		CSF+/PET+	2.29 (0.13)			0.02 (0.01)		
Cognition	MMSE	CSF-/PET-	28.7 (0.7)			-0.03 (0.05)		
		CSF-/PET+	28.4 (0.7)	0.438	0.201	0.09 (0.16)	0.465	**<0.001**
		CSF+/PET-	28.7 (0.7)	0.976	**<0.001**	-0.14 (0.10)	0.298	**<0.001**
		CSF+/PET+	28.0 (0.7)			-0.80 (0.05)		
	Memory composite	CSF-/PET-	1.40 (0.31)			0.00 (0.01)		
		CSF-/PET+	1.53 (0.33)	0.361	**<0.001**	0.00 (0.02)	0.740	**<0.001**
		CSF+/PET-	1.25 (0.32)	0.098	**<0.001**	-0.01 (0.02)	0.603	**<0.001**
		CSF+/PET+	0.83 (0.32)			-0.13 (0.01)		
	Executive functioning composite	CSF-/PET-	2.53 (0.35)			0.03 (0.01)		
		CSF-/PET+	2.68 (0.37)	0.342	**<0.001**	0.03 (0.03)	0.965	**<0.001**
		CSF+/PET-	2.32 (0.36)	**0.034**	**0.010**	0.03 (0.02)	0.835	**<0.001**
		CSF+/PET+	2.07 (0.36)			-0.10 (0.01)		

Results obtained from linear mixed models investigating the effect of amyloid-β CSF/PET discordance on (i) tau pathology assessed by CSF t-tau and p-tau, (ii) tau pathology based on regional [^{18}F]flortaucipir PET, and (iii) cognitive trajectories measured by MMSE, ADNI memory and executive composite scores. Intercept is the main effect of the CSF/PET group and slope is its interaction effect with time. P values show the effect CSF+/PET- or CSF-/PET+ in the model, when concordantly negative (CSF-/PET-) or positive (CSF+/PET+) were chosen as the reference group. CSF tau was assessed at baseline and was followed up a median of 2.0 years later; [^{18}F]flortaucipir PET was first performed a median of 5.2 years after baseline and was followed up a median of 1.3 years later; cognitive tests were assessed at baseline and followed up for a median of 4.2 years.

Table 5. Trajectories for tau pathology and cognition using CSF+/PET+ samples with matching CN/MCI ratios

			Intercept P difference from +/+			Slope P difference from +/+		
			P median	2,5% P quantile	97,5% P quantile	P median	2,5% P quantile	97,5% P quantile
CSF	t-tau	CSF-/PET+	**< 0.0001**	< 0.0001	0.0003	0.1668	0.0490	0.3649
		CSF+/PET-	**< 0.0001**	< 0.0001	< 0.0001	0.5556	0.1769	0.9835
	p-tau	CSF-/PET+	**< 0.0001**	< 0.0001	< 0.0001	0.4187	0.1962	0.6959
		CSF+/PET-	**< 0.0001**	< 0.0001	< 0.0001	0.0839	0.0149	0.2696
[^{18}F]flortaucipir PET	Entorhinal cortex	CSF-/PET+	**< 0.0001**	< 0.0001	0.0002	0.0711	0.0086	0.5642
		CSF+/PET-	**< 0.0001**	< 0.0001	0.0002	0.1770	0.0224	0.9387
	Temporal meta-ROI	CSF-/PET+	**0.0013**	0.0001	0.0133	**0.0396**	0.0055	0.3796
		CSF+/PET-	**< 0.0001**	< 0.0001	0.0008	**0.0164**	0.0014	0.2584
	Braak V and VI	CSF-/PET+	0.2393	0.0641	0.7796	0.0861	0.0197	0.3664
		CSF+/PET-	**0.0006**	< 0.0001	0.0177	**0.0173**	0.0025	0.1361
Cognition	MMSE	CSF-/PET+	0.7854	0.4674	0.9673	**< 0.0001**	< 0.0001	< 0.0001
		CSF+/PET-	0.1039	0.0173	0.4236	**< 0.0001**	< 0.0001	< 0.0001
	Memory composite	CSF-/PET+	**0.0010**	0.0002	0.0038	**< 0.0001**	< 0.0001	< 0.0001
		CSF+/PET-	**0.0229**	0.0034	0.1002	**< 0.0001**	< 0.0001	< 0.0001
	Executive functioning composite	CSF-/PET+	**0.0023**	0.0004	0.0088	**0.0002**	< 0.0001	0.0015
		CSF+/PET-	0.2102	0.0376	0.5825	**< 0.0001**	< 0.0001	< 0.0001

Results obtained from linear mixed models investigating the effect of amyloid-β CSF/PET discordance on (i) tau pathology assessed by CSF t-tau and p-tau, (ii) tau pathology based on regional [^{18}F]flortaucipir PET, and (iii) cognitive trajectories measured by MMSE, ADNI memory and executive composite scores. In order to achieve a similar CN/MCI ratio in the CSF+/PET+ group compared to the CSF-/PET- (49%), CSF+/PET- (49%), and CSF-/PET+ (50%), random samples (100 iterations, without replacement) were drawn from the CSF+/PET+ group, where each sample consisted of the all CN participants of the CSF+/PET+ group (n=79) and randomly drawn n=79/239 MCI CSF+/PET+ participants, resulting in a 50/50 CN/MCI ratio. Presented in the table are the results from the n=100 linear mixed models where in each a random CSF+/PET+ sample was used instead of the total CSF+/PET+ group. The median p values and their quantiles for the intercepts represent the main effect of the CSF/PET group and the slopes represent the interaction between CSF/PET group*time.

Table 6. Longitudinal trajectories for tau pathology and cognition covarying for APOE ε4 status

				P difference from			P difference from	
			Intercept β (SD)	CSF-/PET-	CSF+/PET+	Slope β (SD)	CSF-/PET-	CSF+/PET+
CSF	t-tau	CSF-/PET-	134.3 (32.6)			-0.54 (0.87)		
		CSF-/PET+	136.0 (33.2)	0.832	**<0.001**	4.93 (3.07)	0.087	0.267
		CSF+/PET-	133.9 (33.1)	0.948	**<0.001**	-0.15 (1.77)	0.845	0.421
		CSF+/PET+	170.8 (32.7)			1.40 (0.83)		
	p-tau	CSF-/PET-	69.7 (15.3)			2.35 (0.84)		
		CSF-/PET+	71.9 (15.6)	0.551	**<0.001**	2.78 (3.03)	0.890	0.481
		CSF+/PET-	69.8 (15.6)	0.966	**<0.001**	1.80 (1.74)	0.778	0.096
		CSF+/PET+	91.2 (15.4)			4.99 (0.80)		
[^{18}F]flortaucipir PET	Entorhinal cortex	CSF-/PET-	2.18 (0.18)			0.00 (0.01)		
		CSF-/PET+	2.15 (0.19)	0.540	**<0.001**	-0.01 (0.01)	0.608	0.067
		CSF+/PET-	2.18 (0.18)	0.829	**<0.001**	0.00 (0.01)	0.891	0.151
		CSF+/PET+	2.36 (0.18)			0.02 (0.01)		
	Temporal meta-ROI	CSF-/PET-	2.95 (0.22)			0.00 (0.01)		
		CSF-/PET+	2.95 (0.23)	0.999	**0.012**	0.01 (0.01)	0.575	**0.031**
		CSF+/PET-	2.93 (0.22)	0.761	**<0.001**	0.01 (0.01)	0.618	**0.010**
		CSF+/PET+	3.12 (0.22)			0.04 (0.01)		
	Braak V and VI	CSF-/PET-	2.21 (0.14)			0.00 (0.00)		
		CSF-/PET+	2.25 (0.14)	0.262	0.279	0.00 (0.01)	0.832	0.113
		CSF+/PET-	2.2 (0.14)	0.785	**0.002**	0.00 (0.01)	0.766	**0.022**
		CSF+/PET+	2.3 (0.14)			0.02 (0.01)		
Cognition	MMSE	CSF-/PET-	29.4 (0.7)			-0.02 (0.05)		
		CSF-/PET+	29.2 (0.7)	0.552	0.500	0.10 (0.16)	0.455	**<0.001**
		CSF+/PET-	29.5 (0.7)	0.529	**0.008**	-0.14 (0.10)	0.297	**<0.001**
		CSF+/PET+	29.0 (0.7)			-0.79 (0.05)		
	Memory composite	CSF-/PET-	1.64 (0.32)			0.00 (0.01)		
		CSF-/PET+	1.79 (0.35)	0.288	**<0.001**	0.01 (0.02)	0.737	**<0.001**
		CSF+/PET-	1.54 (0.34)	0.233	**<0.001**	-0.01 (0.02)	0.605	**<0.001**
		CSF+/PET+	1.16 (0.34)			-0.12 (0.01)		
	Executive functioning composite	CSF-/PET-	2.8 (0.36)			0.03 (0.01)		
		CSF-/PET+	2.97 (0.39)	0.274	**0.001**	0.03 (0.03)	0.963	**<0.001**
		CSF+/PET-	2.63 (0.38)	0.096	**0.040**	0.03 (0.02)	0.838	**<0.001**
		CSF+/PET+	2.43 (0.38)			-0.10 (0.01)		

Results obtained from linear mixed models investigating the effect of amyloid-β CSF/PET discordance on (i) tau pathology assessed by CSF t-tau and p-tau, (ii) tau pathology based on regional [^{18}F]flortaucipir PET, and (iii) cognitive trajectories measured by MMSE, ADNI memory and executive composite scores, while covarying for APOE ε4. Intercept is the main effect of the CSF/PET group and slope is its interaction effect with time. P values show the effect CSF+/PET- or CSF-/PET+ in the model, when concordantly negative (CSF-/PET-) or positive (CSF+/PET+) were chosen as the reference group.

Table 7. Progression of syndrome diagnosis

A. From CN to MCI or dementia

Group	Progressors (n)	vs CSF-/PET-				vs CSF+/PET+			
		Co-efficient	Standard error	Hazard ratio	P value	Co-efficient	Standard error	Hazard ratio	P value
CSF-/PET-	18/151	–	–	–	–	-1.10	0.31	0.33[0.18, 0.62]	0.0005
CSF+/PET-	8/39	0.49	0.43	1.64[0.71, 3.77]	0.2457	-0.60	0.41	0.55[0.24, 1.22]	0.1405
CSF-/PET+	2/13	0.22	0.75	1.25[0.29, 5.40]	0.7642	-0.87	0.74	0.42[0.10, 1.77]	0.2359
CSF+/PET+	24/79	1.10	0.31	3.00[1.62, 5.54]	0.0005	–	–	–	–

B. From MCI to dementia

Group	Progressors (n)	vs CSF-/PET-				vs CSF+/PET+			
		Co-efficient	Standard error	Hazard ratio	P value	Co-efficient	Standard error	Hazard ratio	P value
CSF-/PET-	13/155	–	–	–	–	-2.20	0.30	0.11[0.06, 0.20]	<0.0001
CSF+/PET-	3/41	-0.08	0.64	0.92[0.26, 3.25]	0.9027	-2.28	0.59	0.10[0.03, 0.32]	0.0001
CSF-/PET+	0/13	-16.01	2431.92	0[0, Inf]	0.9947	-18.21	2431.92	0[0, Inf]	0.9940
CSF+/PET+	115/239	2.20	0.30	9.01[5.05, 16.08]	<0.0001	–	–	–	–

Output from Cox-regression models investigating the association between amyloid-β CSF/PET profile and progression of syndrome diagnosis for CN participants (to MCI and/or dementia, A), and participants with MCI (progression to dementia, B).

Table 8. Study participants with [^{18}F]flortaucipir PET

	CSF-/PET-	CSF+/PET-	CSF-/PET+	CSF+/PET+
n (%)	123 (49)	34 (13)	16 (6)	80 (32)
Sex, male (%)	68 (55)	16 (47)	5 (31)	40 (50)
Age (mean (SD))	71 (7)^{D}	71 (7)^{D}	69 (5)^{D}	74 (7)^{ABC}
Education (median [IQR])	18 [16, 19]^{D}	16 [16, 18]	16 [14, 18]	16 [14, 18]^{A}
APOE ε4 carriership (%)	27 (22)^{D}	14 (41)	4 (25)	49 (61)^{A}
Diagnosis, MCI (%)	52 (42)	13 (38)	6 (38)	49 (61)
[^{18}F]florbetapir PET composite SUVR (median [IQR])	1.02 [0.98, 1.06]^{BCD}	1.05 [1.01, 1.08]^{ACD}	1.15 [1.13, 1.19]^{ABD}	1.33 [1.23, 1.46]^{ABC}
CSF $A\beta_{42}$ (median [IQR])	235 [212, 249]^{BD}	166 [152, 182]^{ACD}	215 [208, 241]^{BD}	138 [125, 154]^{ABC}
Cognitive test scores (baseline):				
MMSE (mean (SD))	28.9 (1.3)	29.0 (1.1)	28.6 (1.9)	28.2 (1.8)
ADNI memory composite (mean (SD))	0.99 (0.67)^{D}	0.88 (0.62)^{D}	1.22 (0.41)^{D}	0.45 (0.68)^{ABC}
ADNI executive composite (mean (SD))	1.02 (0.85)^{BD}	0.64 (0.57)^{A}	1.14 (0.80)^{BD}	0.41 (0.71)^{AC}
CSF tau measures (baseline):				
CSF t-tau (median [IQR])	56 [43, 68]^{D}	52 [39, 65]^{D}	57 [48, 75]^{D}	96 [62, 139]^{ABC}
CSF p-tau (median [IQR])	27 [22, 35]^{D}	22 [19, 31]^{D}	27 [24, 51]^{D}	46 [33, 66]^{ABC}
[^{18}F]flortaucipir PET (5 years later):				
Entorhinal SUVR (median [IQR])	1.12 [1.06, 1.17]^{D}	1.13 [1.08, 1.19]^{D}	1.11 [1.06, 1.15]^{D}	1.40 [1.21, 1.61]^{ABC}
Temporal meta-ROI SUVR (median [IQR])	1.18 [1.13, 1.23]^{D}	1.18 [1.15, 1.23]^{D}	1.18 [1.16, 1.24]^{D}	1.36 [1.22, 1.58]^{ABC}
BRAAK V and VI SUVR (median [IQR])	1.06 [1.02, 1.10]^{CD}	1.08 [1.01, 1.10]^{D}	1.11 [1.07, 1.17]^{AD}	1.16 [1.06, 1.25]^{ABC}

A,B,C,D indicate differences ($p < 0.05$) from other groups: A – difference from CSF-/PET-; B – difference from CSF+/PET-; C – difference from CSF-/PET+; D – difference from CSF+/PET+. Baseline diagnosis was either cognitively normal or mild cognitive impairment (MCI). Cognitive test scores were compared while adjusting for age, sex and education. False discovery rate (FDR) correction was used to account for multiple comparison.

Table 9. Comparison of the participant characteristics in the total cohort and in the sample with available [18F]flortaucipir PET

	CSF-/PET-			CSF+/PET-			CSF-/PET+			CSF+/PET+		
	Total cohort	With [^{18}F]FTP	p	Total cohort	With [^{18}F]FTP	p	Total cohort	With [^{18}F]FTP	p	Total cohort	With [^{18}F]FTP	p
n(%)	306 (42)	123 (49)		80 (11)	34 (13)		26 (4)	16 (6)		318 (44)	80 (32)	
Sex, male(%)	161 (53)	68 (55)	0.693	48 (60)	16 (47)	0.286	6 (23)	5(31)	0.823	165 (52)	40 (50)	0.860
Age (mean(SD))	72 (7)	71 (7)	0.556	72 (8)	71 (7)	0.266	71 (6)	69 (5)	0.485	74 (7)	74 (7)	0.877
Education (median[IQR])	17 [14,18]	18 [16,19]	0.223	16 [16,18]	16 [16,18]	0.954	16 [14,18]	16 [14,18]	0.751	16 [14,18]	16 [14,18]	0.325
APOE ε4 carriership(%)	53 (17)	27 (22)	0.329	32 (40)	14 (41)	1	8 (31)	4 (25)	0.960	203 (64)	49 (61)	0.765
Diagnosis, MCI(%)	155 (51)	52 (42)	0.143	41 (51)	13 (38)	0.285	13(50)	6(38)	0.638	239(75)	49(61)	**0.019**
[^{18}F]FTP composite SUVR (median[IQR])	1.01 [0.98, 1.04]	1.02 [0.98, 1.06]	0.119	1.05 [1.00, 1.08]	1.05 [1.01, 1.08]	0.482	1.15 [1.13, 1.19]	1.15 [1.13, 1.19]	0.826	1.36 [1.26, 1.49]	1.33 [1.23, 1.46]	0.207
CSF $A\beta_{42}$ (median[IQR])	232 [213, 249]	235 [212, 249]	0.888	164 [149, 181]	166 [152, 182]	0.614	214 [204, 243]	215 [208, 241]	0.876	136 [121, 154]	138 [125, 154]	0.738
Cognitive test scores (baseline):												
MMSE (mean(SD))	28.8 (1.4)	28.9 (1.3)	0.533	28.8 (1.4)	29.0 (1.1)	0.496	28.6 (1.6)	28.6 (1.9)	0.980	28 (1.8)	28.2 (1.8)	0.327
ADNI memory composite (mean(SD))	0.90 (0.68)	0.99 (0.67)	0.238	0.76 (0.64)	0.88 (0.62)	0.361	1.09 (0.48)	1.22 (0.41)	0.348	0.30 (0.71)	0.45 (0.68)	0.070
ADNI executive composite (mean(SD))	0.85 (0.85)	1.02 (0.85)	0.064	0.55 (0.74)	0.64 (0.57)	0.472	1.13 (0.77)	1.14 (0.80)	0.958	0.27 (0.86)	0.41 (0.71)	0.134

Table 9. Continued from previous page

	CSF-/PET-			CSF+/PET-			CSF-/PET+			CSF+/PET+		
	Total cohort	**With [^{18}F]FTP**	**p**	**Total cohort**	**With [^{18}F]FTP**	**p**	**Total cohort**	**With [^{18}F]FTP**	**p**	**Total cohort**	**With [^{18}F]FTP**	p
CSF tau measures (baseline):												
CSF t-tau (median[IQR])	53 [42,68]	56 [43,68]	0.443	55 [39,76]	52 [39,65]	0.499	57 [48,74]	57 [48,75]	0.795	93 [68,135]	96 [62,139]	0.983
CSF p-tau (median[IQR])	25 [20,35]	27 [22,35]	0.294	24 [19,37]	22 [19,31]	0.494	26 [21,43]	27 [24,51]	0.551	47 [35,65]	46 [33,66]	0.692
[^{18}F]flortaucipir PET (5 years later):												
Entorhinal SUVR (median[IQR])	1.12 [1.06, 1.17]	1.12 [1.06, 1.17]	1	1.13 [1.08, 1.19]	1.13 [1.08, 1.19]	1	1.11 [1.06, 1.15]	1.11 [1.06, 1.15]	1	1.40 [1.21, 1.61]	1.40 [1.21, 1.61]	1
Temporal meta-ROI SUVR (median[IQR])	1.18 [1.13, 1.23]	1.18 [1.13, 1.23]	1	1.18 [1.15, 1.23]	1.18 [1.15, 1.23]	1	1.18 [1.16, 1.24]	1.18 [1.16, 1.24]	1	1.36 [1.22, 1.58]	1.36 [1.22, 1.58]	1
BRAAK V and VI SUVR (median[IQR])	1.06 [1.02, 1.10]	1.06 [1.02, 1.10]	1	1.08 [1.01, 1.10]	1.08 [1.01, 1.10]	1	1.11 [1.07, 1.17]	1.11 [1.07,1.17]	1	1.16 [1.06, 1.25]	1.16 [1.06, 1.25]	1

Groups were compared using two-sample t-tests, Wilcoxon tests and two-proportion z-tests.

Table 10. Comparison of the participant characteristics in the total cohort and in the sample with available [18F]flortaucipir PET by random sampling

	CSF-/PET-			CSF+/PET-			CSF-/PET+			CSF+/PET+		
	P med	2,5% P quant	97,5% P quant	P med	2,5% P quant	97,5% P quant	P med	2,5% P quant	97,5% P quant	P med	2,5% P quant	97,5% P quant
Sex, male (%)	0.7013	0.1804	1	0.4661	0.0650	1	1	0.3924	1	0.7517	0.2030	1
Age (mean (SD))	0.5820	0.1022	0.9720	0.3351	0.0522	0.9423	0.5361	0.1344	0.9722	0.7027	0.2535	0.9828
Education (median [IQR])	0.2962	0.0409	0.9583	0.7224	0.2785	0.9925	0.7495	0.2316	0.9927	0.439	0.0571	0.9561
APOE ε4 carriership (%)	0.4212	0.0623	1	1	0.3723	1	1	0.4566	1	0.8703	0.2089	1
Diagnosis, MCI (%)	0.2498	0.0413	0.8498	0.4637	0.0700	1	0.7216	0.2888	1	0.0898	0.0049	0.5090
[^{18}F]FTP PET composite SUVR (median [IQR])	0.1908	0.0235	0.7272	0.5725	0.1205	0.9807	0.7628	0.3452	0.9850	0.3368	0.0415	0.9650
CSF $A\beta_{42}$ (median [IQR])	0.7416	0.1980	0.9851	0.5725	0.1302	0.9860	0.7198	0.254	0.9849	0.5926	0.1160	0.9728
Cognitive test scores (baseline):												
MMSE (mean (SD))	0.6609	0.1532	0.9615	0.5298	0.1057	0.9656	0.7494	0.3365	1	0.5229	0.0615	0.9652
ADNI memory composite (mean (SD))	0.3645	0.0496	0.8894	0.4245	0.0463	0.9462	0.4057	0.1115	0.9774	0.1850	0.0144	0.8253
ADNI executive composite (mean (SD))	0.1477	0.0096	0.7533	0.5755	0.1050	0.9626	0.7752	0.3304	0.9810	0.2446	0.0104	0.8476
CSF tau measures (baseline):												
CSF t-tau (median [IQR])	0.5521	0.1070	0.9749	0.4954	0.0991	0.9392	0.7772	0.3746	0.9929	0.6822	0.2029	0.9683
CSF p-tau (median [IQR])	0.3969	0.0569	0.9883	0.5539	0.1083	0.983	0.6039	0.1962	0.9778	0.6647	0.2033	0.9701
[^{18}F]flortaucipir PET (5 years later):												
Entorhinal SUVR (median [IQR])	0.6749	0.1650	0.9658	0.7561	0.2555	0.9905	0.7326	0.2684	1	0.5645	0.0562	0.9933
Temporal meta-ROI SUVR (median [IQR])	0.6497	0.1793	0.9748	0.6744	0.2381	0.9745	0.7741	0.3103	1	0.5257	0.0800	0.9400
BRAAK V and VI SUVR (median [IQR])	0.6095	0.1972	0.9868	0.6971	0.2006	0.9930	0.7315	0.2563	1	0.5605	0.0788	0.9931

For each amyloid-β CSF/PET subgroup, all participants with available [^{18}F]flortaucipir PET were compared with a random sample from the total cohort (100 iterations, without replacement). The number of participants in each sample equalled the number of participants in the corresponding CSF/PET group with available [^{18}F]flortaucipir PET. In each iteration groups were compared using two-sample t-tests, Wilcoxon tests and two-proportion z-tests where appropriate, and p values (median and quantiles) of these testes are presented.

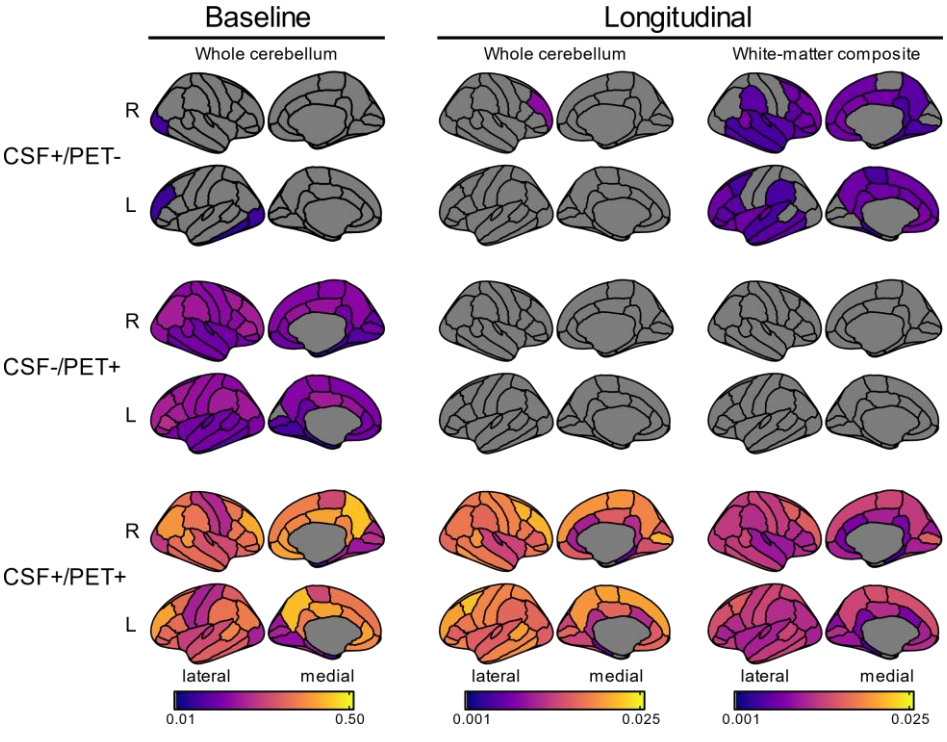


Figure 6. Accumulation of amyloid-β measured by [18F]florbetapir PET in participants with available [18F]flortaucipir PET

Results obtained from linear mixed models, with the colors indicating β-coefficients relative to the CSF-/PET- group. The three sections show the difference between the group of interest (CSF+/PET-, CSF-/PET+ or CSF+/PET+) and CSF-/PET-. The first column shows the β-coefficient for the baseline effect of the CSF/PET group when using whole cerebellum as the reference region. The second and third columns show the β-coefficients for the interaction between CSF/PET group and time as the longitudinal change when using whole cerebellum or composite white matter as the reference, respectively. Only regions with $p<0.05$ are shown. Image was created using the ggseg package in R.

Table 11. Regional [18F]florbetapir uptake of CSF+/PET- vs CSF-/PET- in participants with available [18F]flortaucipir PET

	Whole cerebellum reference				White matter composite reference			
	Intercept β	**p**	**Slope β**	**p**	**Intercept β**	**p**	**Slope β**	**p**
Total composite	0.028 (0.019)	0.147	0.004 (0.003)	0.154	0.028 (0.012)	**0.015**	0.005 (0.002)	**0.001**
Parietal	0.035 (0.022)	0.116	0.003 (0.003)	0.333	0.033 (0.014)	**0.016**	0.004 (0.002)	**0.021**
Temporal	0.018 (0.019)	0.330	0.003 (0.003)	0.230	0.021 (0.012)	0.078	0.004 (0.002)	**0.006**
Cingulate	0.027 (0.022)	0.224	0.006 (0.004)	0.116	0.028 (0.013)	**0.033**	0.006 (0.002)	**0.001**
Frontal	0.034 (0.022)	0.119	0.006 (0.003)	0.089	0.033 (0.013)	**0.015**	0.006 (0.002)	**0.001**

Results obtained from linear mixed models showing regional [^{18}F]florbetapir uptake between CSF+/PET- and CSF-/PET- at baseline (intercept β) and longitudinally (slope β). Composite score standardized uptake values (SUV) were taken from the ANDI database and divided by the whole cerebellar or white matter composite region SUVs to create SUV ratios.

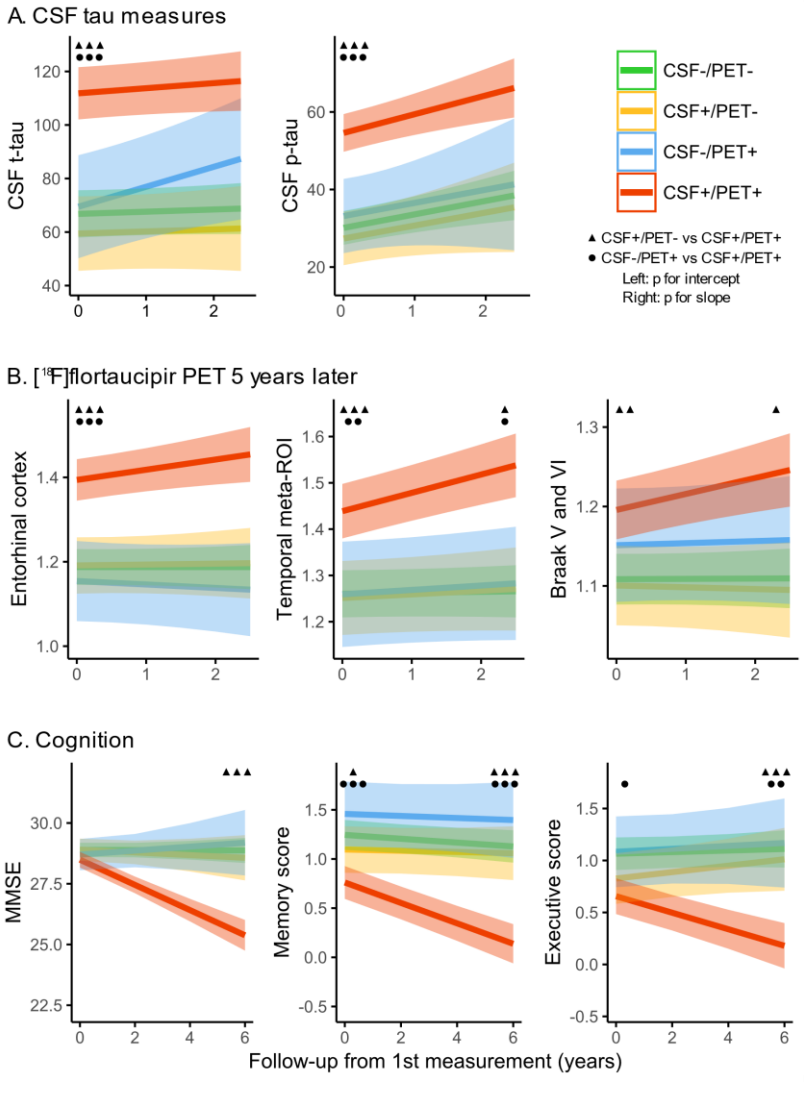


Figure 7. Longitudinal trajectories of tau pathology and cognition in participants with available [18F]flortaucipir PET

Results obtained from linear mixed models investigating the effect of amyloid-β CSF/PET discordance on **A** tau pathology assessed by CSF t-tau and p-tau, **B** tau pathology based on regional [^{18}F]flortaucipir PET, and **C** cognitive trajectories measured by MMSE, ADNI memory and executive composite scores. Difference from CSF+/PET+ is illustrated by black triangles (for CSF+/PET-) or circles (for CSF-/PET+) with the number of symbols indicating statistical difference (one: $p<0.05$; two: $p<0.01$; three: $p<0.001$). Symbols on the left side of a plot show difference in intercept (main effect of CSF/PET group in the model), and symbols on the right side show a difference in slope (interaction between CSF/PET group and time). Image was created using the ggeffects package in R.

Table 12. Longitudinal trajectories for tau pathology and cognition in participants with available [18F]flortaucipir PET

				P difference from			P difference from	
			Intercept β (SD)	CSF-/ PET-	CSF+/ PET+	Slope β (SD)	CSF-/ PET-	CSF+/ PET+
CSF	t-tau	CSF-/PET-	229.2 (56.7)			0.83 (1.15)		
		CSF-/PET+	231.9 (56.6)	0.795	**<0.001**	7.46 (3.29)	0.056	0.119
		CSF+/PET-	221.9 (57.0)	0.332	**<0.001**	0.78 (2.13)	0.986	0.656
		CSF+/PET+	274.3 (56.3)			1.90 (1.42)		
	p-tau	CSF-/PET-	66.8 (28.2)			3.48 (1.07)		
		CSF-/PET+	69.9 (28.2)	0.552	**<0.001**	3.40 (3.16)	0.980	0.676
		CSF+/PET-	64.1 (28.4)	0.458	**<0.001**	3.35 (2.09)	0.955	0.549
		CSF+/PET+	91.3 (28.0)			4.83 (1.32)		
[^{18}F]flor-taucipir PET	Entorhinal cortex	CSF-/PET-	2.27 (0.18)			0.00 (0.01)		
		CSF-/PET+	2.23 (0.18)	0.510	**<0.001**	-0.01 (0.01)	0.617	0.065
		CSF+/PET-	2.27 (0.18)	0.918	**<0.001**	0.00 (0.01)	0.875	0.149
		CSF+/PET+	2.47 (0.18)			0.02 (0.01)		
	Temporal meta-ROI	CSF-/PET-	2.98 (0.22)			0.00 (0.01)		
		CSF-/PET+	2.98 (0.22)	0.986	**0.006**	0.01 (0.01)	0.573	**0.031**
		CSF+/PET-	2.97 (0.22)	0.835	**<0.001**	0.01 (0.01)	0.615	**0.010**
		CSF+/PET+	3.16 (0.21)			0.04 (0.01)		
	Braak V and VI	CSF-/PET-	2.21 (0.14)			0.00 (0.00)		
		CSF-/PET+	2.25 (0.14)	0.260	0.274	0.00 (0.01)	0.832	0.113
		CSF+/PET-	2.20 (0.14)	0.770	**0.002**	0.00 (0.01)	0.765	**0.022**
		CSF+/PET+	2.29 (0.13)			0.02 (0.01)		

Table 12. Continued from previous page

				P difference from			P difference from	
			Intercept β (SD)	CSF-/	CSF+/	**Slope β**	CSF-/	CSF+/
Cognition	MMSE	CSF-/PET-	27.5 (1)	PET-	PET+	**(SD)**	PET-	PET+
		CSF-/PET+	27.3 (1)	0.572	0.551	0.08 (0.12)	0.487	**<0.001**
		CSF+/PET-	27.5 (1)	0.966	0.113	-0.06 (0.08)	0.558	**<0.001**
		CSF+/PET+	27.1 (1)			-0.52 (0.05)		
	Memory composite	CSF-/PET-	0.74 (0.51)			-0.02 (0.01)		
		CSF-/PET+	0.95 (0.52)	0.220	**<0.001**	-0.01 (0.02)	0.695	**<0.001**
		CSF+/PET-	0.59 (0.51)	0.236	**0.013**	-0.01 (0.02)	0.432	**<0.001**
		CSF+/PET+	0.25 (0.52)			-0.10 (0.01)		
	Executive functioning composite	CSF-/PET-	1.34 (0.52)			0.01 (0.01)		
		CSF-/PET+	1.36 (0.53)	0.918	**0.023**	0.01 (0.03)	0.836	**0.004**
		CSF+/PET-	1.10 (0.53)	0.066	0.222	0.03 (0.02)	0.300	**<0.001**
		CSF+/PET+	0.93 (0.53)			-0.08 (0.01)		

Results obtained from linear mixed models investigating the effect of amyloid-β CSF/PET discordance on (i) tau pathology assessed by CSF t-tau and p-tau, (ii) tau pathology based on regional [^{18}F]flortaucipir PET, and (iii) cognitive trajectories measured by MMSE, ADNI memory and executive composite scores. Intercept is the main effect of the CSF/PET group and slope is its interaction effect with time. P values show the effect CSF+/PET- or CSF-/PET+ in the model, when concordantly negative (CSF-/PET-) or positive (CSF+/PET+) were chosen as the reference group.

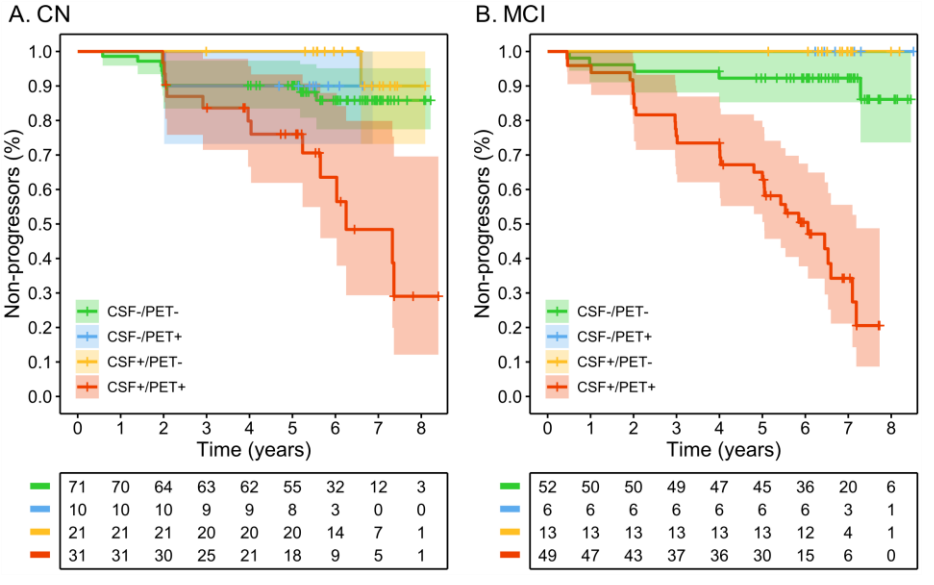


Figure 8. Kaplan-Meier curves for clinical progression for participants with available [18F]flortaucipir PET

Results obtained from the Kaplan-Meier estimate investigating the association between amyloid-β CSF/PET profile and clinical progression for CN participants (progression to MCI or dementia, A) or and participants with MCI (progression to dementia, B). Tables below the figures report per year the number of participants with available follow-up data.

Table 13. Progression of syndrome diagnosis in participants with available [18F]flortaucipir PET

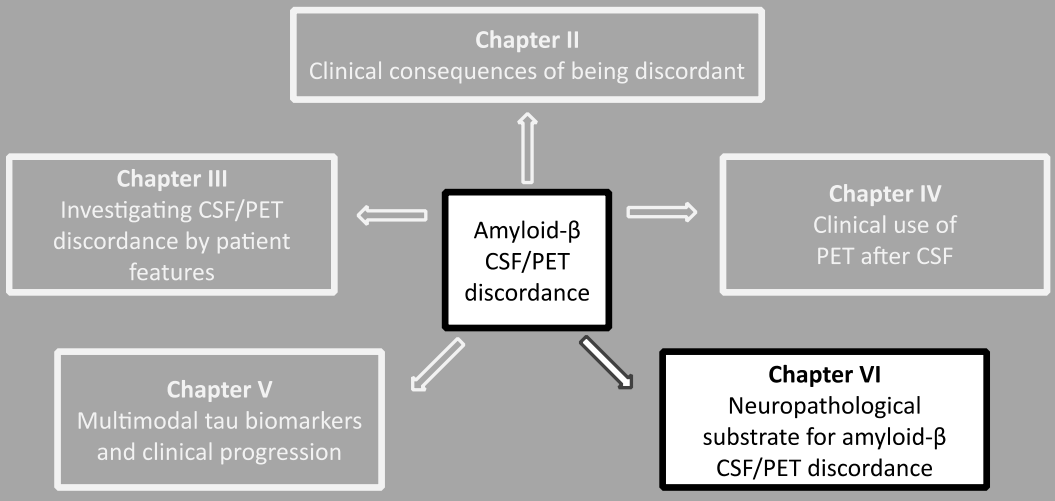
A. From CN to MCI or dementia

Group	Progressors (n)	vs CSF-/PET-				vs CSF+/PET+			
		Co-efficient	Standard error	Hazard ratio	P value	Co-efficient	Standard error	Hazard ratio	P value
CSF-/PET-	9/71	–	–	–	–	-1.28	0.44	0.28[0.12, 0.66]	0.0034
CSF+/PET-	1/21	-1.22	1.05	0.29[0.04, 2.33]	0.2468	-2.50	1.04	0.08[0.01, 0.63]	0.0162
CSF-/PET+	1/10	-0.12	1.06	0.88[0.11, 6.99]	0.9059	-1.40	1.04	0.25[0.03, 1.89]	0.1772
CSF+/PET+	13/31	1.28	0.44	3.60[1.53, 8.48]	0.0034	–	–	–	–

B. From MCI to dementia

Group	Progressors (n)	vs CSF-/PET-				vs CSF+/PET+			
		Co-efficient	Standard error	Hazard ratio	P value	Co-efficient	Standard error	Hazard ratio	P value
CSF-/PET-	5/52	–	–	–	–	-2.30	0.49	0.10[0.04, 0.26]	<0.0001
CSF+/PET-	0/13	-17.49	5584.95	0[0, Inf]	0.9975	-19.79	5584.95	0[0, Inf]	0.9972
CSF-/PET+	0/6	-17.52	7803.89	0[0, Inf]	0.9982	-19.82	7803.89	0[0, Inf]	0.9980
CSF+/PET+	29/49	2.30	0.49	9.98[3.79, 26.26]	<0.0001	–	–	–	–

Output from Cox-regression models investigating the association between amyloid-β CSF/PET profile and progression of syndrome diagnosis for CN participants (to MCI and/or dementia, A), and participants with MCI (progression to dementia, B).


Chapter II
Clinical consequences of being discordant
Chapter III
Investigating CSF/PET discordance by patient features
Amyloid-β CSF/PET discordance
Chapter IV
Clinical use of PET after CSF
Chapter V
Multimodal tau biomarkers and clinical progression
Chapter VI
Neuropathological substrate for amyloid-β CSF/PET discordance

CHAPTER VI: Amyloid-β PET and CSF in an autopsy-confirmed cohort

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ABSTRACT

Objective: Accumulation of amyloid-β is among the earliest changes in Alzheimer's disease (AD). Amyloid-β positron emission tomography (PET) and $A\beta_{42}$ in cerebrospinal fluid (CSF) both assess amyloid-β pathology *in-vivo*, but 10-20% of cases show discordant (CSF+/PET- or CSF-/PET+) results. The neuropathological correspondence with amyloid-β CSF/PET discordance is unknown.

Methods: We included 21 patients from our tertiary memory clinic who had undergone both CSF $A\beta_{42}$ analysis and amyloid-β PET, and had neuropathological data available. Amyloid-β PET and CSF results were compared with neuropathological ABC scores (comprising of Thal (A), Braak (B) and CERAD (C) stage, all ranging from 0 [low] to 3 [high]) and neuropathological diagnosis.

Results: Neuropathological diagnosis was AD in 11 (52%) patients. Amyloid-β PET was positive in all A3, C2 and C3 cases and in one of the two A2 cases. CSF $A\beta_{42}$ was positive in 92% of ≥A2 and 90% of ≥C2 cases. PET and CSF were discordant in 3/21 (14%) cases: CSF+/PET- in a patient with granulomatosis with polyangiitis (A0B0C0), CSF+/PET- in a patient with FTLD-TDP type B (A2B1C1), and CSF-/PET+ in a patient with AD (A3B3C3). Two CSF+/PET+ cases had a non-AD neuropathological diagnosis, that is FTLD-TDP type E (A3B1C1) and adult-onset leukoencephalopathy with axonal spheroids (A1B1C0).

Interpretation: Our study demonstrates neuropathological underpinnings of amyloid-β CSF/PET discordance. Furthermore, amyloid-β biomarker positivity on both PET and CSF did not invariably result in an AD diagnosis at autopsy, illustrating the importance of considering relevant co-morbidities when evaluating amyloid-β biomarker results.

INTRODUCTION

Among the earliest neuropathological events in Alzheimer's disease (AD) is the accumulation and aggregation of amyloid-β, which occurs decades before symptom onset.[1] Amyloid-β can aggregate in the brain parenchyma as plaques or in the cerebral vasculature as cerebral amyloid angiopathy (CAA). Two methods are currently employed to assess amyloid-β pathology *in-vivo*. $A\beta_{42}$ levels in the cerebrospinal fluid (CSF) reflect the concentrations of soluble amyloid-β, which has been shown to correlate with amyloid-β deposits in the brain.[2] Alternatively, positron emission tomography (PET) with amyloid-β radiotracers can be used to visualize cerebral amyloid-β deposits.[3–5] These two methods are considered interchangeable for the assessment of amyloid pathology *in-vivo* and for the diagnosis of AD in both clinical practice and research.[6,7]

In the majority of cases amyloid-β PET and CSF are concordant, but in 10-20% of patients they show discordant (CSF+/PET- or CSF-/PET+) results.[8,9] One possible hypothesis for the amyloid-β CSF/PET discordance is that soluble CSF $A\beta_{42}$ decreases before significant fibrillar amyloid-β deposits can be detected by PET.[10,11] Although studies have been performed to compare either amyloid-β PET or CSF $A\beta_{42}$ to neuropathological examination results,[2–5] so far no head-to-head cohort studies have been performed to compare *in-vivo* amyloid-β CSF and PET results to neuropathological findings. Previously, two case reports of patients with discordant amyloid-β CSF/PET (both CSF+/PET-) and available neuropathology have been published,[12,13] in which the negative PET signal was attributed to the lack of neuritic plaques at autopsy. Further investigating the correspondence between amyloid-β PET, CSF and neuropathology ("standard of truth") is important to shed light on the underlying cause of amyloid-β CSF/PET discordance. Also, if CSF+/PET- amyloid-β status is an indicator of early amyloid-β pathology, this could be instrumental for future disease modifying therapies.[14] Therefore, the aim of this study was to investigate the concordance between PET and CSF amyloid-β status in a sample with available neuropathological results and to characterize the amyloid-β CSF/PET discordant cases neuropathologically.

METHODS

Participants

We retrospectively included 21 autopsy cases from the Amsterdam Dementia Cohort who had undergone both CSF $A\beta_{42}$ analysis and amyloid-β PET during life. Patients visiting our tertiary memory center are screened according to a standardized protocol,[15] including a clinical and neuropsychological evaluation, *APOE* genotyping, magnetic

resonance imaging (MRI), and a lumbar puncture (LP) for CSF biomarker analysis. Clinical diagnosis is determined during a multidisciplinary meeting.

Amyloid-β PET and CSF analyses were performed between 2007 and 2016 and neuropathological diagnosis was performed between 2011 and 2019 (**Figure 1**). In this sample, LP for CSF analysis always preceded amyloid-β PET and, the median CSF-PET time was 28 [interquartile range (IQR): 18, 56] days. The median time difference between amyloid-β PET and patient death was 3.0 (IQR: 1.7, 6.5) years and the time difference between LP and patient death was 3.3 (IQR: 2.0, 6.7) years.

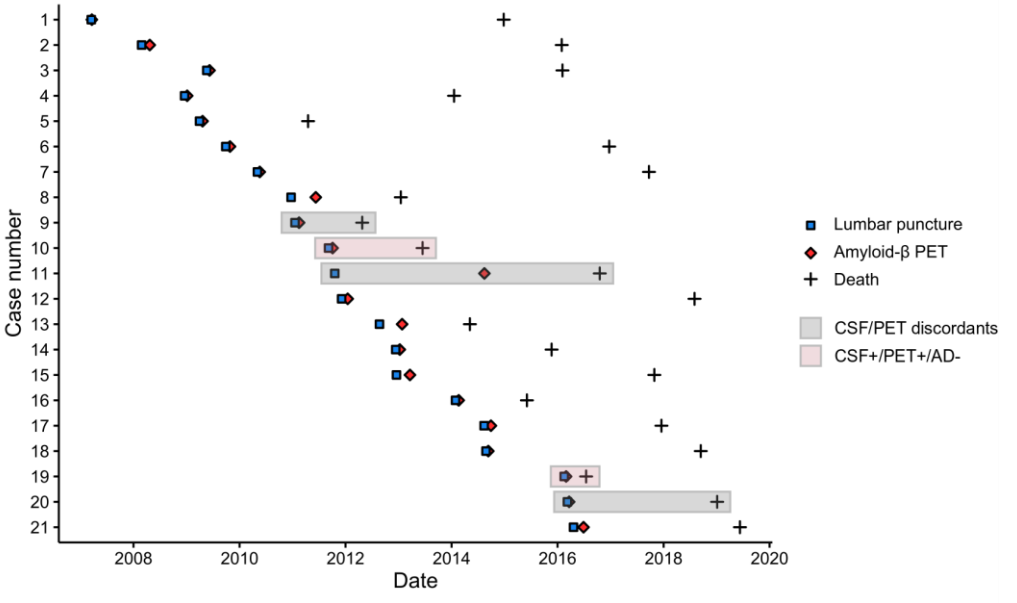


Figure 1. Time between lumbar puncture, amyloid-β PET and patient death.

Abbreviations: AD Alzheimer's disease, CSF cerebrospinal fluid, PET positron emission tomography.

Cerebrospinal fluid

CSF was obtained during life by LP between L3/4 and L5/S1, using a 25-gauge needle and a syringe.[16] Samples were collected in polypropylene collection tubes and centrifuged at 1800g for 10 min at 4°C and thereafter frozen at -20 °C until routine biomarker analysis. Manual analyses of $A\beta_{42}$, total tau (t-tau) and phosphorylated tau (p-tau) were performed using sandwich ELISAs (Innotest assays: β-amyloid1-42, tTAU-Ag and PhosphoTAU-181p; Fujirebio) in the Neurochemistry Laboratory of the Department of Clinical Chemistry of Amsterdam UMC. If two CSF results were

available, we used the result closest to the amyloid-β PET (3 cases, all with concordant $A\beta_{42}$ status between two samples). As median CSF $A\beta_{42}$ values of our cohort have been gradually increasing over the years, we were unable to use the original CSF $A\beta_{42}$ values.[17] Therefore, we used CSF $A\beta_{42}$ values that have been adjusted for the longitudinal upward drift with a uniform cut-off of 813 pg/ml (<813 pg/mL considered as CSF amyloid-β positive).[18] Additionally, as it has been previously shown that the ratio of CSF $A\beta_{42}$ with CSF (p)tau is superior to CSF $A\beta_{42}$ in predicting the diagnosis of AD,[19] we also used a CSF p-tau/$A\beta_{42}$ ratio with a previously validated cut-off of 0.054.[20] This cut-off was obtained by mixture modelling of 2711 CSF results of the Amsterdam Dementia Cohort, similar to previous work.[18]

Amyloid-β positron emission tomography

Amyloid-β PET was performed using the following PET scanners: ECAT EXACT HR+ scanner (Siemens Healthcare, Germany), Gemini TF PET/CT, Ingenuity TF PET-CT and Ingenuity PET/MRI (Philips Medical Systems, the Netherlands). We included fifteen cases with [^{11}C]Pittsburgh Compound B (PIB),[21] three with [^{18}F]florbetaben[22] and three with [^{18}F]flutemetamol.[23] Amyloid-β PET status (positive or negative) was determined by a majority visual read of three reads. All scans were initially read by an expert nuclear medicine physician (BvB, from 2007 to 2016, read 1). In addition, in 2019 the scans were reread for this study by BvB (read 2) and LC (with extensive experience in reading amyloid-β PET scans, read 3), while being blinded to the results of other visual reads, CSF, and neuropathological results. The three amyloid-β PET visual reads were concordant (either +/+/+ or -/-/-) in 18/21 cases. In the three remaining cases (nr 5, 10, 19), two of the three visual reads were positive, and as such these cases were considered PET-positive.

Neuropathology

Autopsies were performed by the Department of Pathology of Amsterdam UMC; location VUmc for the Netherlands Brain Bank or for VUmc. Brain donors or their next of kin signed informed consent regarding the usage of brain tissue and clinical records for research purposes. Brain autopsies and neuropathological diagnosis were performed according to international guidelines of Brain Net Europe II consortium (http://www.brainnet-europe.org) and the applicable diagnostic criteria.[24,25] For this particular study, every case also without suspicion of AD pathology during life, was scored by AR and BB for AD neuropathological changes according to the ABC scoring system by AR and BB,[24] in which the A stands for amyloid-β Thal phase,[26] B for Braak stage for neurofibrillary tangles,[1] and C for CERAD criteria for neuritic plaques.[27] When present, CAA was classified as Type 1 (including capillaries in the parenchyma) or Type

2 (leptomeningeal/cortical without capillary involvement) and staged according to Thal et al.[28]

Statistical analysis

Statistical analysis was conducted using R software (Version 3.6.1). We used descriptive statistics to characterize the sample. We used the Cochrane-Armitage trend test to examine the associations between amyloid-β biomarkers and the neuropathological ABC scores,[29] which allowed us to compare both PET and CSF to neuropathology as we had only binarized results available for amyloid-β PET.

RESULTS

Study population

In our sample of 21 cases, 16 (76%) were male and 10 (48%) were carriers of an *APOE* ε4 allele **(Table 1)**. Mean age at death was 65±8 years and the average last known Mini-Mental State Examination (MMSE, median 2.0 years before death) was 20±6. Eleven (52%) patients had a clinical diagnosis of AD, which was in accordance with neuropathological diagnosis in all AD cases. Two cases (4 and 15, both CSF/PET concordant) carried an autosomal dominant mutation associated with AD. In 15 (71%) cases CAA (11 CAA-Type 1, 4 CAA-Type 2) was observed at neuropathological examination.

In vivo amyloid-β status

Thirteen (62%) cases were defined as amyloid-β positive based on PET, 14 (67%) based on CSF $A\beta_{42}$ and 11 (52%) based on CSF p-tau/$A\beta_{42}$ ratio. In our sample, CSF $A\beta_{42}$ and amyloid-β PET were concordant in 18 (86%) cases. CSF p-tau/$A\beta_{42}$ ratio was concordant with amyloid-β PET in 17 (81%) cases and with CSF $A\beta_{42}$ in 16 (76%) cases.

Discordance between amyloid-β PET, CSF and neuropathological diagnosis

Of the three discordant cases between CSF $A\beta_{42}$ and amyloid-β PET, two were CSF+/PET- (case 9, clinical diagnosis: frontotemporal dementia [neuropathological diagnosis: frontotemporal lobar degeneration (FTLD)-TDP type B, ABC score: A2B1C1] and case 20 with vasculitis [granulomatosis with polyangiitis, A0B0C0], and

Table 1. Case characteristics

	Nr	Sex	Age at death	APOE genotype	Clinical diagnosis	CSF $A\beta_{42}$	Amyloid-β PET	CSF/PET status	Neuropathology ABC	Neuropathology Primary diagnosis	Neuropathology CAA-Type
	1	m	75	E4E4	AD	810	positive	CSF+/PET+	A3B3C3	AD	2
	2	m	65	E3E3	AD	640	positive	CSF+/PET+	A3B3C3	AD	2
	3	m	65	E3E3	CBS	940	negative	CSF-/PET-	A0B1C0	FTLD-TDP type A	-
	4	f	43	E3E3	AD	554	positive	CSF+/PET+	A3B3C3	AD	1
	5	f	64	E4E4	AD	619	positive	CSF+/PET+	A2B2C2	AD	1
	6	m	69	E3E4	AD	504	positive	CSF+/PET+	A3B3C3	AD	1
	7	m	76	E3E4	FTD	1110	negative	CSF-/PET-	A1B0C0	FTLD-TDP type A	-
	8	m	60	-	Dementia unspecified	1136	negative	CSF-/PET-	A0B0C0	Autoimmune encephalitis	-
*	9	m	75	E3E3	FTD	787	negative	CSF+/PET-	A2B1C1	FTLD-TDP type B	-
†	10	m	64	E4E4	SD	739	positive	CSF+/PET+	A3B1C1	FTLD-TDP type E	1
*	11	m	68	E3E3	AD	828	positive	CSF-/PET+	A3B3C3	AD	2
	12	m	68	E3E3	Dementia unspecified	1167	negative	CSF-/PET-	A0B1C0	LBD	-
	13	m	65	E2E3	FTD	1708	negative	CSF-/PET-	A1B1C0	FTLD/MND TDP type B	1
	14	m	70	E3E4	AD	755	positive	CSF+/PET+	A3B3C3	AD	2
	15	f	62	E4E4	AD	681	positive	CSF+/PET+	A3B3C3	AD	1
	16	f	61	E3E4	FTD	862	negative	CSF-/PET-	A1B0C0	FTLD-TDP type E	1
	17	m	73	E3E4	AD	644	positive	CSF+/PET+	A3B2C1	AD	1
	18	m	53	E3E3	AD	587	positive	CSF+/PET+	A3B3C3	AD	1
†	19	m	50	E3E3	HDLS	676	positive	CSF+/PET+	A1B1C0	Leukodystrophy due to HDLS	1
*	20	f	65	E3E3	Vasculitis	646	negative	CSF+/PET-	A0B0C0	Granulomatosis with polyangiitis	-
	21	m	65	E3E4	AD	397	positive	CSF+/PET+	A3B3C3	AD	1

Asterisks (*) in the first column highlight CSF/PET discordant cases and crosses (†) highlight CSF+/PET+ cases with a non-AD neuropathological diagnosis. Values under 813 pg/mL for CSF $A\beta_{42}$ are pathological. Amyloid-β PET positivity was determined by majority visual read. Neuropathological ABC scoring system entails amyloid-β Thal (A) phase, Braak (B) stage for neurofibrillary tangles and CERAD (C) criteria for neuritic plaques. CAA column indicates the neuropathological type of cerebral amyloid angiopathy: Type 1 (capillary) or Type 2 (leptomeningeal/cortical). Abbreviations: AD Alzheimer's disease, CAA cerebral amyloid angiopathy, CBS corticobasal syndrome, CSF cerebrospinal fluid, FTD frontotemporal dementia, FTLD frontotemporal lobar degeneration, HDLS Adult-onset leukoencephalopathy with axonal spheroids, LBD Lewy body dementia, MND motoneuron disease, PET positron emission tomography, SD semantic dementia.

one was CSF-/PET+ (case 11 with AD [AD, A3B3C3], **Figure 2A-C**). The three amyloid-β CSF/PET discordant patients all had an *APOE* ε3/ε3 genotype. In addition, there were two CSF+/PET+ cases with a non-AD primary neuropathological diagnosis, i.e. case 10 with semantic dementia [FTLD-TDP type E, A3B1C1; CAA-Type 1 stage 2]; and case 20 with adult-onset leukoencephalopathy with axonal spheroids (HDLS) [leukodystrophy due to HDLS, A1B1C0; CAA-Type 1 stage 1] (**Figure 2D-E**).

Association between biomarkers and ABC scores

CSF $A\beta_{42}$ **(Figure 3A)** was positive in 12 of 13 (92%) and CSF p-tau/$A\beta_{42}$ ratio **(Figure 3B)** in 10 of 13 (77%) of the A2/A3 cases. Both CSF $A\beta_{42}$ and p-tau/$A\beta_{42}$ ratio were positive in 10 of 11 (91%) B2/B3 cases and 9/10 (90%) C2/C3 cases. Amyloid-β PET **(Figure 3C)** was positive in one of the two A2 cases, and in all A3 and/or B2/B3 and/or C2/C3 cases. Cochrane trend analyses showed that there is an increasing proportion of biomarker-positive cases from score 0 to 3 across all ABC scores for amyloid-β PET (Z-score=-3.93 for A, Z=-3.81 for B, Z=-3.68 for C, all $p < 0.001$) and CSF $A\beta_{42}$ (Z= -2.92, p=0.003 for A; Z=-2.46, p=0.014 for B; Z=-2.60, p=0.009 for C). In *APOE* ε4 carriers, both CSF $A\beta_{42}$ and amyloid-β PET were positive in all A2/A3 and/or B2/B3 and/or C2/C3 cases. In *APOE* ε4 non-carriers, CSF $A\beta_{42}$ was positive in 80% of A2/A3, 75% of B2/B3 and 75% of C2/C3 cases, and amyloid-β PET was positive in 80% A2/A3 cases and all B2/B3 and/or C2/C3 cases.

Association between biomarkers and neuropathological diagnosis

Finally, we investigated the association between binarized biomarker results and neuropathological diagnosis. Amyloid-β PET was positive in all AD cases, but also indicated amyloid-β pathology in two cases without AD as neuropathological diagnosis **(Figure 4)**. Both CSF $A\beta_{42}$ and p-tau/$A\beta_{42}$ were positive in 10 of 11 AD cases. Decreased CSF $A\beta_{42}$ with a normal CSF p-tau/$A\beta_{42}$ ratio was seen in three non-AD cases (HDLS [A1B1C0]. FTLD-TDP type B [A2B1C1], FTLD-TDP type E [A3B1C1]) and one AD case (A3B3C3). There were three cases with a non-AD neuropathological diagnosis (HDLS, FTLD-TDP type E, FTLD/MND-TDP type B) with normal levels of CSF p-tau but with increased CSF t-tau.

DISCUSSION

The primary aim of this study was to investigate the concordance between PET and CSF amyloid-β status in a sample with available neuropathological results in order to enhance our understanding of the amyloid-β CSF/PET discordant cases. We found that

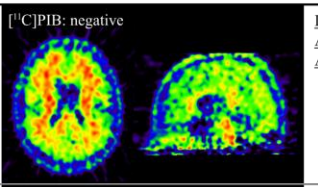
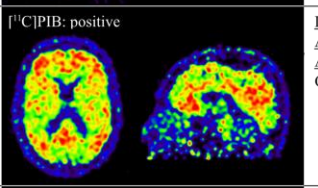
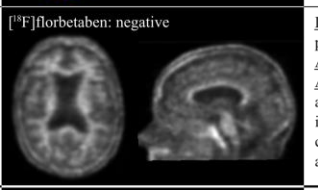
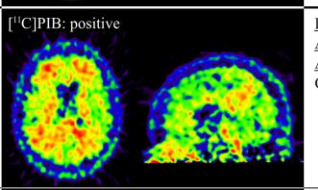
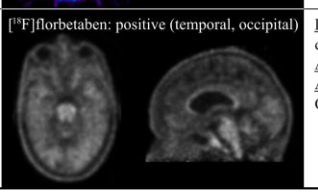
	Clinical findings	CSF	Amyloid-β PET	Neuropathology	Explanations for discord
A case 9	74 y/o man was referred for compulsive and anti-social character change. MMSE was 24/30. On MRI, left hippocampal atrophy (MTA 2), and extensive white matter damage (Fazekas III) with two possible small lacunes were seen. Amyloid-β PET was performed for research and was negative. The patient was diagnosed with possible bvFTD.	**$A\beta_{42}$: 787** t-tau: 279 p-tau: 41		Diagnosis: FTLD-TDP type B ABC score: A2B1C1 Additional findings: N/A	**CSF+/PET-/AD-** > CSF detected amyloid co-patholog > CSF $A\beta_{42}$ could be false-positive a close to the cut-off (individual CSF production or pre-analytical factors)
B case 11	63 y/o man visited our center with memory complaints. MMSE was 30/30 but the neuropsychological exam confirmed memory dysfunction. The MRI was normal and the CSF analysis was inconclusive. Amnestic MCI was diagnosed. During follow-up there was a slow decline in memory and executive functioning and after 3 years amyloid-β PET was requested. The PET scan was read positive and the patient was diagnosed with AD.	$A\beta_{42}$: 828 **t-tau: 498** **p-tau: 66**		Diagnosis: AD ABC score: A3B3C3 Additional findings: CAA-Type 2 (stage 1)	**CSF-/PET+/AD+** > CSF $A\beta_{42}$ is false negative as it is c to the cut-off and CSF tau levels wer already increased (individual variati of CSF $A\beta_{42}$ or pre-analytical factors > The long time difference between analysis and amyloid PET could be a additional contributing factor
C case 20	62 y/o woman was referred because of apathy and tiredness. MMSE was 30/30. White-matter lesions and a lacune in the right thalamus were found on the MRI. Aβ PET was performed and was negative. CSF pleocytosis was found and $A\beta_{42}$ was decreased. An autoimmune cause was suspected. Over the next 2 years there was subacute cognitive decline and progression of vascular damage based on MRI. FDG PET showed increased uptake in the thoracic aorta, indicative of vasculitis.	**$A\beta_{42}$: 646** t-tau: 251 p-tau: 40		Diagnosis: Granulomatosis with polyangiitis ABC score: A0B0C0 Additional findings: Aβ-positive axons in the substantia nigra and in the sub-thalamic nucleus, corresponding to leakage of amyloid precursor protein.	**CSF+/PET-/AD-** > Decreased CSF $A\beta_{42}$ due to neuroinflammation
D case 10	53 y/o man was referred because of severe word finding problems and behavioral change. MMSE was 18/30. Family history was positive for early-onset dementia. The CSF analysis was considered normal (prior adjustment for $A\beta_{42}$ longitudinal drift) and MRI showed anteriotemporal atrophy (MTA: left 2, right 1). FDG PET showed bilateral frontal and left temporal hypometabolism. Aβ PET was clinically requested and initially read as negative. Patient was diagnosed with SD.	**$A\beta_{42}$: 739** t-tau: 362 p-tau: 38		Diagnosis: FTLD-TDP type E ABC score: A3B1C1 Additional findings: CAA-Type 1 (stage 2)	**CSF+/PET+/AD-** > Detection of amyloid co-pathology by both CSF and PET > Possible detection of CAA
E case 19	49 y/o man was referred to our memory clinic due to multi-domain (memory, executive functioning, visuospatial, behavioral) cognitive decline over 1-2 years. MMSE was 24/40. Familial history was positive for early-onset dementia. MRI showed extensive parietal atrophy and white matter lesions. Amyloid-β PET was requested for research and was initially read as negative. Genetic analysis found CSF1R mutation and HDLS was diagnosed.	**$A\beta_{42}$: 676** **t-tau: 528** p-tau: 33		Diagnosis: Leukodystrophy due to HDLS ABC score: A1B1C0 Additional findings: CAA-Type 1 (stage 1)	**CSF+/PET+/AD-** > Detection of CAA by PET and CS

Figure 2. Discordance between amyloid-β CSF, PET and autopsy.

(Figure on previous page) Vignettes illustrating amyloid-β CSF/PET discordant cases (A,B,C) and CSF+/PET+ cases with a non-AD neuropathological diagnosis (D,E). CSF values for $A\beta_{42}$ <813 pg/mL, for phosphorylated tau (p-tau) >52 pg/mL, and for total tau (t-tau) >375 pg/mL are pathological (indicated by bold). Amyloid-β PET scans in cases 10 and 19 were initially read as amyloid-negative, but for this study the scans were considered amyloid-positive based on majority visual read. Abbreviations: AD Alzheimer's disease, CAA cerebral amyloid angiopathy, CSF cerebrospinal fluid, FDG Fluorodeoxyglucose, FTD frontotemporal dementia, FTLD frontotemporal lobar degeneration, HDLS Adult-onset leukoencephalopathy with axonal spheroids, MCI Mild cognitive impairment, MMSE Mini-Mental State, Examination, MRI Magnetic resonance imaging, MTA - Medial temporal lobe atrophy, PET positron emission tomography, SD sematic dementia, TDP transactive response DNA binding protein.

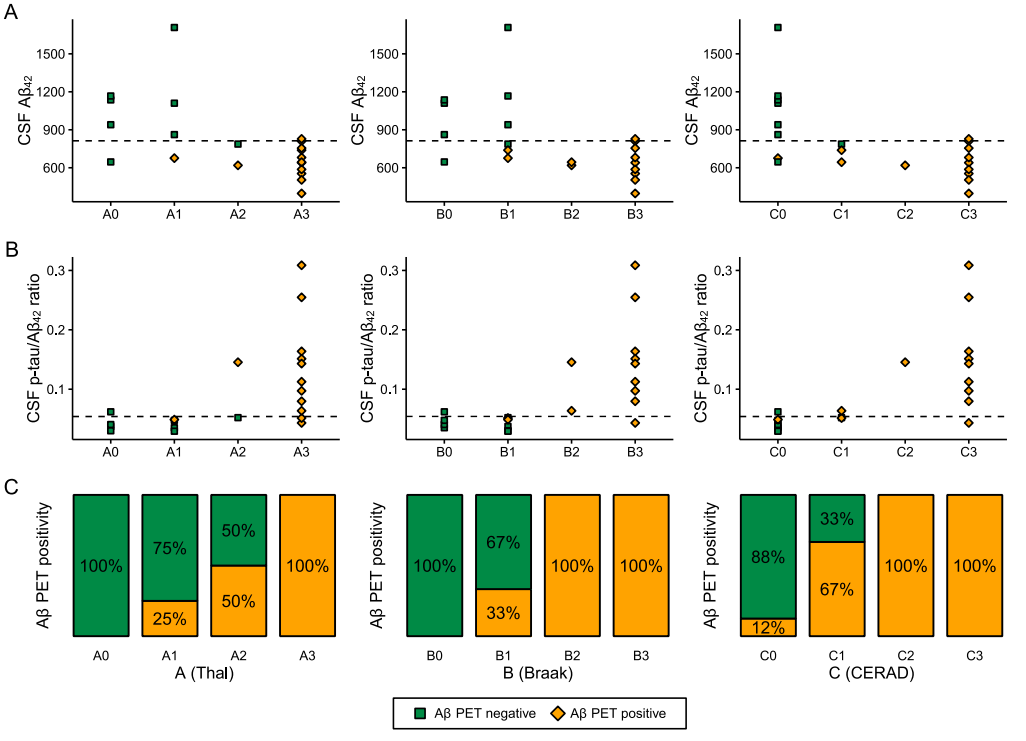


Figure 3. Correspondence of CSF $A\beta_{42}$ (A), CSF p-tau/$A\beta_{42}$ ratio (B), and amyloid-β (Aβ) PET (C) to neuropathological ABC scoring.

Neuropathological ABC scoring system entails amyloid-β Thal phase (A0-A3), Braak stage for neurofibrillary tangles (B0-B3) and CERAD criteria for neuritic plaques (C0-C3). Dashed lines represent cut-offs for CSF $A\beta_{42}$ (813 pg/mL) and CSF p-tau/$A\beta_{42}$ ratio (0.054).

although both CSF and PET generally captured AD pathological change, there was still 14% (3/21) discordance between the two modalities. In our sample, possible reasons for amyloid-β CSF/PET discordance included neuroinflammation (CSF+/PET- in a case

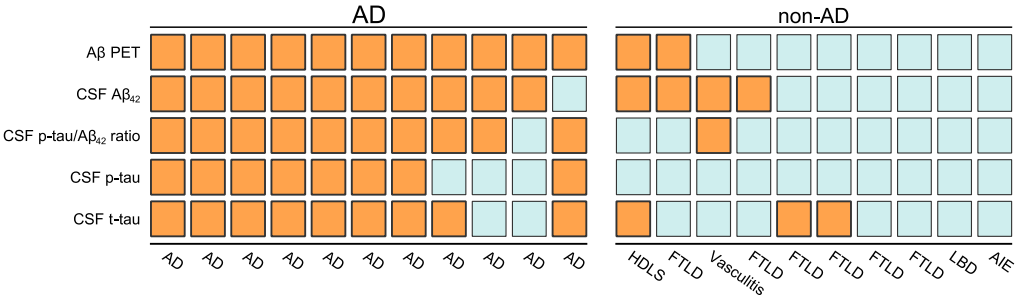


Figure 4. Biomarker status by primary neuropathological diagnosis.

Colors indicate binarized status of biomarkers: orange for biomarker-positive, blue for biomarker-negative. Abbreviations: Aβ Amyloid-β, AD Alzheimer's disease, AIE autoimmune encephalitis, CSF cerebrospinal fluid, FTLD frontotemporal lobar degeneration, HDLS Adult-onset leukoencephalopathy with axonal spheroids, PET positron emission tomography.

of granulomatosis with polyangiitis, A0B0C0), detection of amyloid-β co-pathology (CSF+/PET- in FTLD-TDP type B, A2B1C1) and additional factors influencing CSF $A\beta_{42}$ levels (CSF-/PET+ in AD, A3B3C3). Additionally, we described two CSF+/PET+ non-AD cases illustrating that amyloid-β biomarker positivity on both PET and CSF does not invariably result in an AD diagnosis at autopsy. This highlights that it is important to consider other comorbidities when evaluating the results of amyloid-β biomarkers, especially since molecular biomarkers for non-AD neurodegenerative diseases are currently lacking.

Although in the majority of cases, amyloid-β PET and CSF $A\beta_{42}$ show concordant results, 10-20% discordant CSF/PET status has repeatedly been shown.[8,9,30] As amyloid-β CSF/PET discordance rates are highest in patients with early disease, it has been hypothesized that CSF/PET discordance might be partly explained by early decreases of CSF $A\beta_{42}$ that precede amyloid-β depositions visible by PET.[10,11] On the other hand, amyloid-β CSF/PET discordance in patients with dementia could be explained by one modality detecting beginning amyloid-β co-pathology in non-AD cases.[8] To our knowledge, this is the first serial study including patients who have both amyloid-β PET and CSF $A\beta_{42}$ in addition to neuropathological data available. In line with previous *in vivo* studies, we found a 14% (3/21) CSF/PET discordance rate. We reported a CSF+/PET- patient with A2B1C1 FTLD-TDP type B, where it is feasible that the reduction of CSF $A\beta_{42}$ is caused by concomitant amyloid-β pathology. However, as the $A\beta_{42}$ value was relatively close to the cut-off, it is not possible to entirely exclude individual CSF $A\beta_{42}$ dynamics (i.e. this patient intrinsically producing less $A\beta_{42}$)[31] or pre-analytical factors.[32] Previously, two CSF+/PET- case reports with available neuropathology have been published. First, a negative PIB PET scan was reported in

a 91-year-old patient with abnormal CSF $A\beta_{42}$ and tau biomarkers with sporadic AD.[12] The negative amyloid-β PET status was attributed to the absence of a significant amount of fibrillar plaques (i.e. with a fibrillar core that the tracer binds to), although diffuse plaques were present. Second, low PIB PET retention with decreased CSF $A\beta_{42}$ was reported in a familial AD case with arctic amyloid precursor protein (APP) mutation, thought to be caused by the lack of fibrillar amyloid-β plaques characteristic for this mutation.[13] Future studies with neuropathological data are needed to further validate whether amyloid-β CSF+/PET- status is caused by beginning amyloid-β depositions and explore additional neuropathological substrates for CSF/PET discordance, such as differences in distribution, load and morphology of amyloid-β plaques and possible influences of co-pathologies.

In our sample, there were two cases with amyloid-β CSF+/PET+ biomarker status who did not meet neuropathological criteria for AD. The first had a diagnosis of FTLD-TDP type E with a high Thal score but only sparse neuritic plaques (A3B1C1). It is feasible that in this case both biomarkers detected concomitant amyloid-β co-pathology as increased PIB PET signal has been shown to be related to fibrillar plaque load even in case of sparse neuritic plaques.[33,34] The patient was also diagnosed with CAA-Type 1 stage 2, which could also contribute to the amyloid-positivity, as CAA has been shown to affect both amyloid-β PET tracer uptake[35] and CSF $A\beta_{42}$ levels.[36] The second CSF+/PET+ patient with a low score for AD pathology (A1B1C0) was diagnosed with HDLS, an autosomal dominant white matter disease due to mutations in the gene encoding colony stimulating factor 1 receptor (CSF1R).[37] Previous case reports of HDLS including CSF analyses have provided no evidence for alterations in $A\beta_{42}$ levels.[38,39] It is also unlikely that pre-analytical assay effects caused the decrease of CSF $A\beta_{42}$ in this case as the patient had a separate CSF $A\beta_{42}$ sample with decreased $A\beta_{42}$ 4 months earlier. Similar to the previous patient, CAA-Type 1 was present and might have contributed to the positive amyloid-β biomarker status, especially since PET tracer uptake was seen predominantly in the occipital region, a predilection site for CAA pathology.[35] This illustrates that even concordant positivity of two amyloid-β biomarkers does not always result in a neuropathological diagnosis of AD, and relevant co-pathologies should always be considered.

There were four cases where CSF $A\beta_{42}$ was decreased without a neuropathological diagnosis of AD. In three of them there was neuropathological evidence for amyloid-β co-pathology, but we also we described a CSF+/PET- case with granulomatosis with polyangiitis (formerly known as Wegener's granulomatosis), which is in line with literature, as neuroinflammation[40,41] as well as infection[42,43] have been previously shown to cause decreased CSF $A\beta_{42}$ without presence of AD pathology. This highlights that in select cases, there might be unspecific decreases in CSF $A\beta_{42}$ levels without AD, although these cases might be distinguished from AD pathology based on clinical findings and MR imaging. In our particular case, after Aβ immunostaining, Aβ

immunoreactive axons were seen, which can be attributed to the leakage of APP that is reported in various conditions such as ischemia, traumatic brain injury and - similar to this case – inflammation [44]. The possible connection of this finding with the decrease of CSF $A\beta_{42}$ is unclear, although it is tempting to hypothesize that the loss of APP leads to the interruption of the APP pathway and the reduction of its product $A\beta_{42}$ in the CSF. We were unable to find previous case reports of vasculitis with available CSF $A\beta_{42}$ analysis, but primary angiitis of the central nervous system has been associated with decreased APP in the CSF[45], lending support to that speculative theory.

CSF p-tau/$A\beta_{42}$ ratio was slightly more specific than CSF $A\beta_{42}$ for capturing the neuropathological diagnosis of AD, which has been previously shown in studies involving living subjects.[19] In the CSF-/PET+ discordant case we presented, the patient with a clinically advanced AD dementia had a CSF $A\beta_{42}$ value just above the cut-off, but CSF p-tau/$A\beta_{42}$ ratio was in the pathological range. In this case, CSF $A\beta_{42}$ was likely false-negative, possibly due to individual differences in CSF dynamics, as both CSF t-tau and p-tau were already increased. This also highlights the advantage of using continuous measurements as opposed to binarized data, as the distance from cut-off includes additional information. Although (p)tau/$A\beta_{42}$ ratio may be superior to $A\beta_{42}$ when predicting clinically advanced disease with increased (p)tau levels, this may hamper the detection of merely amyloid-β pathology, where tau tangle pathology has not yet begun. This may become clinically significant if anti-amyloid treatment arrives in the future. Finally, we reported an isolated increase of CSF t-tau with normal CSF p-tau levels in three non-AD cases (two FTLD, one HDLS). Although CSF t-tau and p-tau are highly correlated, this finding supports the notion that CSF t-tau can increase in other brain pathologies[46] and CSF p-tau is more AD-specific.[47]

The primary strength of our study is the availability of two amyloid-β biomarkers and a neuropathological assessment in a relatively large patient cohort that allowed us to compare the two *in vivo* amyloid-β biomarkers to neuropathological change. Although PET and CSF were usually performed close in time, there was a median 3-year delay between the amyloid-β biomarkers and autopsy, as is often the case with studies involving *in-vivo* biomarkers and autopsy data. While this might have impacted our results, a major change over three years is unlikely, given the remarkably slow course of AD.[48] We used standardized uptake value ratio images for PET visual read, which could have an impact on our results as non-displaceable binding potential images have been shown to be more reliable in detecting early amyloid-β pathology.[49,50] Another limitation is that we included subjects from the year 2006, and over time technologic advancement has taken place, leading to both increased image quality of PET scans and understanding of pre-analytical factors influencing CSF (leading to longitudinal drift of median values, in our cohort). Finally, correcting CSF $A\beta_{42}$ values with CSF $A\beta_{40}$ has been shown to account for the individual variation in the production of amyloid-β.[51] As

CSF $A\beta_{40}$ values were only available for seven patients (and none of them were among the discordant cases), we did not include a $A\beta_{42}/_{40}$ ratio in our analyses.

In conclusion, our findings illustrate a range of reasons for the amyloid-β CSF/PET discordance, and that even concordant amyloid-β biomarker positivity accurately reflecting amyloid-β pathology does not always equal a definite neuropathological diagnosis of AD. Thus, it is important to consider co-morbidities as well as other neurodegenerative diseases when using amyloid-β biomarkers for clinical diagnosis, especially since molecular biomarkers for non-AD neurodegenerative diseases are currently lacking.

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Authors' contributions: JR, RO, FB and PS conceived the study and designed the protocol. JR performed statistical analysis, analyzed/interpreted data and drafted the manuscript. RO and FB provided overall study supervision. JR, BB, LC, RO, FB participated in writing the manuscript. LC and BvB did the visual reads of the amyloid-β PET scans. BB and AR were responsible for neuropathological evaluation. CT, AR, BvB, PS had a major role in the acquisition of data, and critically revised and edited the manuscript for intellectual content. All authors read and approved the final version of the manuscript.

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CHAPTER VII. Summary and discussion

In this thesis we investigated whether the two most widely used amyloid-β modalities (i.e. PET and CSF) provide partially independent information using a variety of approaches - both *in vivo* and *ex vivo*. The main findings of this thesis are:

1. Patients with discordant amyloid-β CSF/PET status are intermediate to CSF-/PET- and CSF+/PET+ groups on genetic (*APOE* ε4 carriership) and CSF (tau) markers of AD, and have similar cognitive trajectories as CSF-/PET- cases.
2. There are subtle differences between the predictive patterns for amyloid-β status based on PET and CSF, suggesting that CSF and PET do not provide identical information.
3. A mismatch between the primary clinical diagnosis and CSF Aβ/tau biomarkers leads to requesting amyloid-β PET in a memory clinic.
4. Discordant amyloid-β CSF/PET groups have lower levels of CSF (p)tau and higher cognitive test scores at baseline and less tau PET signal 5 years later than CSF+/PET+ patients, suggesting discordant amyloid-β CSF/PET is associated with a better prognosis.
5. Amyloid-β CSF+/PET- convert to CSF+/PET+ before tau PET deposition reaches supra-threshold levels, suggesting that CSF+/PET- status marks early stage amyloid-β pathology.
6. Neuroinflammation, detection of amyloid-β co-pathology by CSF in other neurodegenerative diseases, and other factors influencing CSF $A\beta_{42}$ levels can cause amyloid-β CSF/PET discordance in a neuropathologically confirmed sample.

In the section below, a summary is provided for each chapter of this thesis. Thereafter, potential implications of our work are discussed, followed by methodological considerations, future perspectives and the conclusion.

SUMMARY

We started our investigation by exploring amyloid-β CSF/PET discordance in the Amsterdam Dementia Cohort (ADC). For both the analyses presented in **chapter II** and **III**, we included all patients from the ADC with available amyloid-β PET and CSF biomarker analysis in one year. This resulted in a large mixed sample of 768 patients, consisting of subjective cognitive decline (SCD), mild cognitive impairment (MCI), AD and non-AD dementia. We found that in this sample, 13% (n=97) of the participants were CSF/PET discordant, of whom two thirds (n=65) were CSF+/PET- and one third (n=32) were CSF-PET+.

In **chapter II**, we investigated the clinical consequences of having discordant amyloid-β CSF/PET status. We compared various clinical and neurobiological features between discordant (combined CSF+/PET- and CSF-/PET+) groups with CSF-/PET- and CSF+/PET+ groups. We found that CSF/PET discordance rate differed by disease stage, being highest in SCD and non-AD dementia, and lower in AD dementia. Patients with discordant amyloid-β CSF/PET status were intermediate to CSF-/PET- and CSF+/PET+ groups on genetic (*APOE* ε4 carriership) and CSF (tau) markers of AD, and additionally for diagnostic progression, indicating that discordant amyloid status is not benign. We also utilized linear mixed models to investigate the longitudinal cognitive trajectories of these patient groups. In patients without dementia, discordant cases performed similar to the CSF-/PET- group in memory function and global cognition, while the CSF+/PET+ group had a steeper cognitive decline. In patients with dementia (AD and non-AD neurodegenerative disorders combined), however, amyloid-β biomarker discordance or concordance did not affect cognitive trajectories, possibly because the impact of amyloid on cognitive decline is less pronounced at later disease stages in both AD and non-AD with amyloid-β co-pathology.

In **chapter III** we expanded upon the previous work, by focusing on the potential differences of the CSF+/PET- and CSF-/PET+ participants to identify factors that may contribute to amyloid-β CSF/PET discordance. In this study we performed parallel random forest and logistic regression models predicting separately PET and CSF amyloid status using various patient features, such as demographics, *APOE* ε4 carriership, CSF (p)tau, cognitive performance, and MRI visual ratings. This was based on the assumption that if there are significant differences in the predictive patterns of the two modalities, they must convey partially independent information. We found that although *APOE* ε4, CSF tau and p-tau were the most significant predictors for positive amyloid-β status for both PET and CSF, the overall predictive pattern of patient features differed between the modalities, also varying by disease stage, indicating that PET-CSF discordance contains unique information. CSF tau proteins consistently showed a stronger association with amyloid-β status on PET, suggesting that PET might be more specific to advanced AD pathology. In addition, in SCD we found stronger

associations of both *APOE* ε4 carriership and worse memory z-scores with CSF suggesting that CSF might be sensitive early in the disease course. Altogether, this work suggests that various patient features are partly differently associated with amyloid-β positivity on CSF and PET.

We then explored why occasionally amyloid-β PET-scans are clinically requested when CSF $A\beta_{42}$ analysis had already been performed **(chapter IV)**. The rationale for such clinical decision-making was unknown as these two modalities are considered interchangeable in clinical guidelines. We included all such cases from the ADC and performed patient chart reviews to identify factors which led to requesting amyloid-β PET after CSF biomarkers analysis. In this sample, patients were relatively young, often had an atypical presentation of AD and often showed a change in diagnosis. The main reason for requesting an amyloid-β PET scan after performing CSF biomarkers was the occurrence of a mismatch between the primary clinical diagnosis and CSF Aβ/tau biomarkers, implying that this clinical practice took place in complicated clinical dilemmas where diagnostic confidence was low. We additionally rated case-by-case accordance with previously published appropriate-use-criteria[1] (AUC) for amyloid-β PET. The results indicate that sometimes clinical practice was not covered by the AUC, most often when previous CSF analysis did not support current clinical diagnosis. which led to requesting amyloid-β PET. Based on this finding, the PET AUC could possibly be supplemented with the criterion of patients clinically diagnosed with AD without an AD-like CSF biomarker signature.

In **chapter V,** we investigated the association between discordant CSF/PET amyloid-β biomarkers, tau pathology and clinical progression in 730 participants of the Alzheimer's Disease Neuroimaging (ADNI) cohort. In this sample, [^{18}F]flortaucipir tau PET was performed at a median of 5 years after baseline assessment (including amyloid-β CSF/PET), allowing us to measure tau pathology at a considerably later timepoint. We used linear mixed modelling to study whether discordant CSF/PET groups differed from the CSF-/PET- or the CSF+/PET+ participants. Although amyloid-β CSF+/PET- participants showed longitudinal accumulation of amyloid-β based on PET, they had less tau 5 years later than CSF+/PET+ patients, similar to CSF-/PET-. Similarly, discordant amyloid-β status was associated with better cognitive outcome and a lower risk of clinical progression than CSF+/PET+, confirming findings from **chapter II**. These results suggest that CSF+/PET- amyloid-β status reflects a significantly earlier stage of AD CSF+/PET+, and is associated with a distinctly better prognosis for at least 5 years. Participants from the CSF-/PET+ group, however, did not show amyloid accumulation over time nor significant tau pathology or cognitive decline as compared to the CSF-/PET- group. We also investigated whether isolated amyloid-β positivity in CSF is followed by significant tau deposition already at this stage, or whether it will follow more advanced amyloid-β pathology, which is already detectable by both modalities. Based on findings from CSF+/PET- participants several

years later, amyloid-β load detectable by both CSF and PET seems to precede substantial tau deposition.

Finally, in **chapter VI**, we investigated the neuropathological basis of the amyloid-β CSF/PET discordance. To that end, we included 21 autopsy cases from the ADC who had undergone both CSF $A\beta_{42}$ analysis and amyloid-β PET during life. We found that although both CSF and PET generally captured AD pathological change, there was still 14% discordance between the two modalities. Reasons for amyloid-β CSF/PET discordance included reduced CSF $A\beta_{42}$ levels due to neuroinflammation in a case of granulomatosis with polyangiitis, detection of low/moderate amyloid-β co-pathology by CSF in FTLD-TDP type B, and other factors influencing CSF $A\beta_{42}$ levels, such as $A\beta_{42}$ dynamics or pre-analytical factors, causing CSF $A\beta_{42}$ to be negative in a case of advanced AD. Additionally, we described two CSF+/PET+ cases who were classified as non-AD at autopsy, illustrating that amyloid-β biomarker positivity on both PET and CSF does not equal a neuropathological diagnosis of AD. This finding highlights the importance of considering other comorbidities when evaluating amyloid-β biomarker results.

Overall, our findings provide insight into the clinical and pathophysiological consequences of having discordant amyloid-β CSF/PET biomarkers.

GENERAL DISCUSSION AND IMPLICATIONS

Proportion of discordant CSF/PET cases

Although in the majority of the cases amyloid-β PET and CSF showed concordant results, there was a remarkably similar CSF/PET discordance rate ranging from 13% in **chapter II** and **III**, 14% in **chapter VI** to 15% in **chapter V**, in line with previous work from others.[2–4] In **chapter II** we also showed that when removing cases within 10% of the $A\beta_{42}$ cut-off, the discordance expectedly decreased (to 9%), but it did not disappear, suggesting that the CSF/PET discordance cannot just be explained by variation around the cut-off.[3]

In **chapter IV**, there was a higher CSF/PET discordance rate (44%), which could be partly explained by the inherent bias in this sample, as amyloid-β PET was mostly clinically requested in case of a borderline CSF biomarker analysis. Additionally, to reflect clinical decision-making we used the original CSF $A\beta_{42}$ results, which were not adjusted for the longitudinal upwards drift of the median values seen in our cohort.[5,6] Therefore, these values might not best describe the underlying amyloid-β pathology.

Prognostic implications

Previously, it was shown in ADNI data that amyloid-β CSF+/PET- participants had a similar decline in memory to CSF-/PET-, whereas CSF+/PET+ was associated with worse outcomes.[7] In **chapter II** we found similar results using ADC data with a median of 2 year follow-up time. We showed that in pre-dementia the combined discordant group performed similar to CSF-/PET- in memory function and global cognition, while CSF+/PET+ group had a steeper decline. In **chapter V**, we replicated the above-mentioned findings in ADNI with longer follow-up periods (median 4 years), finding similar results for both CSF+/PET- and CSF-/PET- groups. These findings suggest that amyloid-β discordant patients with SCD and MCI have comparable cognitive trajectories compared to CSF-/PET- patients during the first 4 years. This might suggest that group-wise amyloid-β CSF/PET discordance reflects earlier amyloid-β pathology than CSF+/PET+, and therefore might not yet be associated with cognitive decline.

Moreover, in **chapter II** we showed that in patients with dementia (AD and non-AD syndromes combined), amyloid-β biomarker status did not affect cognitive trajectories. It is possible that the effect of amyloid-β on cognitive decline is less pronounced at later disease stages in AD. Also, in non-AD dementia the cognitive decline is most likely caused by the primary pathology, and the amyloid-β positive status reflecting low amyloid-β co-pathology might not have a large effect at this stage.

CSF+/PET- as a marker for early amyloid-β pathology

Previously it has been hypothesized that amyloid-β CSF+/PET- status marks the pathological beginnings of amyloid-β accumulation.[7–9] This could be conceptually explained as CSF $A\beta_{42}$ starting to decrease when it is accumulating into brain tissue, but a certain amyloid-β load is required to create a signal detectable by PET. This was first theorized after the finding that there were more CSF+/PET- participants compared to the CSF-/PET+ group.[8] This higher prevalence of CSF+/PET- has been reported in the majority of studies investigating CSF/PET concordance,[2] especially in recent years. A 2-to-1 CSF+/PET- to CSF-/PET+ ratio was also found in our work in **chapters II / III**, **V** and **VI**. If CSF/PET discordance was only a result of random variation, one would expect a roughly 50:50 ratio of the two groups.

CSF+/PET- group has been reported to be more prevalent in earlier stages comparing to AD dementia, supporting its association with early amyloid pathology.[3] Similarly, in **chapter II** we found that both the overall CSF/PET discordance as well as the CSF+/PET- to CSF-/PET+ ratio were highest in SCD and non-AD dementia, possibly relating to the low amount of amyloid-β (co-)pathology.

In **chapter V** we found that amyloid-β CSF+/PET- participants in ADNI had significantly more accumulation of amyloid-β based on PET compared to the CSF-/PET- group, confirming previous findings in the same cohort using slightly different methodologies to determine PET positivity.[7,9] Longitudinal accumulation of amyloid-β in CSF+/PET- was previously seen in regions associated with early amyloid pathology, i.e. orbitofrontal cortex, anterior and posterior cingulate, and precuneus.[9] Subtle regional differences between CSF+/PET- and CSF-/PET- were present at baseline, illustrating that increased PET signal is already present at the subthreshold range. Similar regional differences between CSF-/PET- and CSF+/PET- were confirmed cross-sectionally in another cohort, leading to creation of regional amyloid-β PET staging system.[10]

We also found in **chapter V** that CSF+/PET- was not associated with higher CSF (p)tau levels at baseline, worse cognitive trajectories nor higher tau PET signal 5 years later. Similarly, in **chapter II**, CSF+/PET- had comparable (p)tau levels compared to CSF-/PET-. Previously, CSF+/PET- was also shown to have comparable glucose metabolism based on FDG PET and longitudinal rates of hippocampal atrophy compared to CSF-/PET-.[7,9] In fact, based on our findings from CSF+/PET- participants 6 years later, CSF+/PET- convert to CSF+/PET+ before tau PET deposition reaches threshold levels, indicating that CSF+/PET- status marks early amyloid-β pathology where significant neuronal loss has not yet begun.

In **chapter III**, we found stronger associations of both APOE4 carriership and worse memory scores with CSF-amyloid in SCD compared to PET-amyloid suggesting that CSF-amyloid might be sensitive early in the disease course. Also, we found that CSF

t-tau and p-tau had a stronger association with PET, similar to previous work.[3] It has been shown that CSF tau biomarker levels start rising before amyloid-PET positivity and reach threshold levels after amyloid-β PET positivity.[10,11] Therefore this finding is in line with the concept of CSF+/PET- being a marker for early AD pathology, as increased CSF (p)tau levels are more likely to occur in the more advanced CSF+/PET+ stage than in CSF+/PET-.

Finally, in **chapter VI** we showed a CSF+/PET- case with early/moderate AD pathology at autopsy. As it is a sole example, it must be interpreted with caution, but it could be considered support for CSF detecting earlier pathology than PET.

There are other findings directly or indirectly supporting the argument that CSF+/PET- is a marker for early amyloid-β pathology. Using cross-sectional CSF data from five CSF assays, it was estimated that CSF $A\beta_{42}$ levels started decreasing at sub-threshold amyloid-β PET SUVR levels although the PET data were analyzed with a global ROI which may have affected it's sensitivity.[10] In a longitudinal study involving cognitively normal amyloid-negative participants, those who progressed to suprathreshold PET amyloid-β status had lower baseline CSF $A\beta_{42}$ levels, and the decrease of CSF $A\beta_{42}$ already started during middle age.[12,13] In familial AD, it has been shown that CSF $A\beta_{42}$ levels started to decrease 25-20 years before estimated symptom onset, whereas significant amyloid-β PET deposition occurred 5-10 years later.[14] When comparing to amyloid-β PET signal levels expressed in centiloids (CL), it has been shown that 12 CL was an optimal cut-off for detecting CSF $A\beta_{42}$ decrease,[15] similar to the level shown to exclude AD pathology at autopsy.[16,17] Usually higher CL values in the 25-30 range values have been shown to best depict the PET visual read threshold,[17,18] although lower CL values up to CL of 12 have been shown in other studies.[16,19] Finally, it has been reported that CSF p-tau levels might increase before positive tau PET reaches threshold levels,[11,20] supporting the notion that soluble changes in the CSF can precede substantial accumulation of pathological protein in the brain in a high enough amount to be visualized by PET. Overall the results of our work with findings from others suggest that CSF+/PET- amyloid-β status is a marker for an early stage of amyloid-β pathology.

Other causes for CSF+/PET-

There are likely other biological factors associated with CSF+PET- status. In **chapter VI**, we presented a CSF+/PET- case of granulomatosis with polyangiitis with no AD neuropathological changes at autopsy. The decrease of CSF $A\beta_{42}$ in such a case is possibly caused by the downregulation or interruption of the APP pathway and similar decrease of $A\beta_{42}$ has been shown in other cases with central nervous system infection[21,22] or neuroinflammation.[23,24] It has also been shown that normal-pressure

hydrocephalus (NPH) is associated with the decrease of CSF proteins (including $A\beta_{42}$), possibly as increased pressure leads to reduction of interstitial space thereby restricting clearance of extracellular fluid.[25–27] This implicates that in some cases, the decrease of CSF $A\beta_{42}$ might be related to other diseases, but these cases are probably distinguishable from AD pathology based on clinical findings and/or MR imaging. There have also been case reports of patients with CSF+/PET- amyloid-β status (both familial[28] and sporadic[29] AD), in which the negative PET signal was attributed to the absence of a significant amount of typical fibrillar plaques at autopsy. Finally, a proportion of CSF+/PET- cases is likely caused by individuals intrinsically producing less $A\beta_{42}$ (resulting in false-positive CSF results) andby methodological variation (further discussed in the Methodological Considerations section).

The heterogeneous nature of CSF-/PET+

In **chapter V** we showed that ADNI participants in the CSF-/PET+ group did not accumulate amyloid-β over time, and had similar low levels of tau pathology (both by CSF at baseline and tau PET 5 years later) and prognosis compared to CSF-/PET- group. However, in **chapter III**, CSF-/PET+ status was associated with higher CSF tau and worse MMSE and memory scores, compared to the CSF-/PET- group. There are several possible reasons for these conflicting results. Most importantly, in **chapter III**, we included patients from the more heterogenous ADC, also including patients with dementia (including non-AD dementia). For **chapter V**, we included participants without dementia from the more homogenous ADNI cohort. In addition, amyloid-β positivity was determined based on semi-quantitative measures in **chapter V**, but it was based on visual read in **chapter III**. Finally, the CSF assays were different in ADNI and ADC.

These results seem to indicate that the amyloid-β CSF-/PET+ group is heterogeneous in nature. First, a proportion of CSF-/PET+ subjects might not have true underlying amyloid-β pathology, and the PET+ status (especially when based on semi-quantitative measures) could be attributed to various factors, such as spill-over tracer signal from the white matter, movement artifacts or processing errors.[3] Similarly, due to the binarized nature of PET visual read, in some equivocal cases a patient might become PET positive based on a single region which may be missed by semi quantitative measures based on a global ROI. In addition, CSF-/PET+ status might be caused by CSF being false-negative due to various methodological factors or due to the cut-off being not able to capture the pathologic changes in case of a high intrinsic production of $A\beta_{42}$. The autopsy proven CSF-/PET+ case from **chapter VI** also suggests that factors influencing CSF $A\beta_{42}$ levels might cause CSF false negativity even in case of advanced amyloid-β pathology. It is likely, that the higher the PET tracer quantitative uptake or the more advanced amyloid-β pathology based on visual read, the more probable it is that PET+ status indicates true pathology. Similarly, the farther away CSF

$A\beta_{42}$ is from the cut-off, the higher the probability that the normal $A\beta_{42}$ levels represent lack of amyloid-β pathology.

PET and advanced AD

It has been previously shown that compared to CSF $A\beta_{42}$, amyloid-β PET positivity is better associated with cognitive function,[30,31] AD diagnosis,[31] hippocampal atrophy,[30] and is a better predictor of cognitive decline [32] and progression of syndrome diagnosis.[31] Similarly, in **chapter III** we found that CSF tau and p-tau were more strongly linked to amyloid-β PET positivity in line with previous work.[3] This different association between CSF/PET and other AD hallmark changes is possibly caused by two factors.

First, this is likely caused by some subjects with isolated CSF $A\beta_{42}$ positivity not yet reaching later stages of the disease within relatively short follow-up time, whereas amyloid-β PET positivity is reached later in the preclinical disease course more closer to atrophy and cognitive decline. An alternative interpretation could be that these isolated $A\beta_{42}$ positivity cases were false positive. Second, it has also been shown that PET and CSF have a non-linear relationship,[33] as there is a lack of continuous decrease of CSF $A\beta_{42}$ in advanced amyloid-β pathology. It has been estimated that the rate of decrease for CSF $A\beta_{42}$ peaks 4 years before the onset of AD dementia, thereafter slowly leading to a plateau.[34] Therefore, unlike amyloid-β PET, CSF $A\beta_{42}$ levels do not capture accumulation of amyloid-β at the later stages. This is linked with the concept that CSF reflects the current ratio of production and clearance of $A\beta_{42}$ whereas PET reflects the net accumulation of amyloid-β.[35] However, at the latest stages amyloid-β PET can also reach a plateau,[36] possibly due to decreased production of amyloid-β as neurons die, reduced clearance of previously deposited amyloid-β as the brain atrophies or changes in the structure of amyloid-β binding sites.[35]

CSF/PET discordance and other amyloidoses

Not much is known whether PET/CSF discordance is associated with other amyloid-β pathologies. For example, it has been shown that amyloid-β PET signal is increased in cerebral amyloid angiopathy (CAA), with a predilection toward occipital regions,[37] which are not involved in the visual read criteria but are usually scored. In the CSF, CAA is associated with decreases of both $A\beta_{40}$ and $A\beta_{42}$ levels.[38] The amyloid-β pathology associated with Down's syndrome consists of mostly diffuse plaques and involves mostly the striatium[39] and PET tracers are more affine to the fibrillar amyloid structure.[40,41] The CSF $A\beta_{42}$ levels are decreased in Down's syndrome[42] and have been shown to precede amyloid-β PET positivity.[43] Finally, after brain trauma, it has been

shown that there is an acute marked increase in CSF $A\beta_{42}$[44] and amyloid-β plaques can rapidly appear.[45] Although these plaques are mostly diffuse, they are also shown to have PET tracer uptake.[46]

Similarly, little information is available about the CSF/PET status in other brain amyloidoses. Possible CSF/PET discordance could depend on the type and structure of amyloid deposition. In prion-related cerebral amyloidosis, for example in sporadic Creutzfeldt-Jakob disease, there might be decrease of CSF $A\beta_{42}$ due to epitope masking,[47,48] and low-affinity PET tracer uptake is possible.[49] In transthyretin-related cerebral amyloidosis there has been shown to be PET tracer uptake[50] but light-chain amyloidomas have been shown not to be associated with tracer uptake.[51] However, further investigation is challenging as other brain amyloidoses are rare.

CSF/PET discordance – clinician's perspective

Even though CSF/PET discordance is associated with better outcomes comparted to CSF+/PET+, our results show that this status is not entirely benign. For example, discordant groups in **chapter II/III** and **V** had higher proportions of *APOE* ε4 carriership compared CSF-/PET-, indirectly hinting to the greater risk of these patients of developing amyloid-β pathology. Interestingly, in **chapter II** we showed that although discordant CSF/PET patients without dementia did not show worse cognitive trajectories compared to concordant negative patients, this status was associated with worse progression of syndrome diagnosis. We also showed that when concordant positive status most often led to an AD diagnosis, this was less often the case in patients with amyloid-β discordant status. These findings illustrate that amyloid-β CSF/PET discordance constitutes a clinical dilemma, and most likely is associated with worse diagnostic confidence.

Amyloid-β PET after CSF

It is evident that in everyday clinical practice patients with discordant amyloid-β CSF/PET status are rare and it is not cost-effective to routinely perform both amyloid-β PET and CSF on the same patient. For the majority of the subjects included in our work, one or both of the modalities were performed in a research setting to determine the utility of *in vivo* amyloid-β diagnostics.[52–56] However, in **chapter IV** we only included cases with clinically requested PET scans and we found that in a tertiary memory clinic setting sometimes amyloid-β PET was requested by the clinician after CSF biomarker analysis. This happened most often when there is a mismatch between the primary clinical diagnosis and CSF biomarker results and was in line with previous reports.[57,58]

This suggests that sometimes having both amyloid-β biomarkers might be warranted as clinicians are becoming more reliant on biomarker results.

In a few cases, this clinical practice was not in accordance with previously published AUC for amyloid-β PET,[1] which advocates use in three groups most likely to benefit based on the clinical findings: patients with an atypical clinical presentation or mixed etiology, persistent unexplained MCI, and unexplained dementia in young patients. Our results argue in favor of including patients with clinical diagnosis conflicting with biomarker results (especially in case of clinical AD diagnosis without an AD-like CSF biomarker signature) to be included in a next update of the PET AUC.

Are amyloid-β PET and CSF interchangeable?

In our work, we set out to find possible differences between amyloid-β PET and CSF and our results suggest, that PET and CSF are not always interchangeable. However, it is important to note that both of these biomarkers were concordant in the vast majority of cases and both reflect neuropathological AD change with good accuracy.[40,59–62] Similarly, in **chapter VI** we found that both CSF and PET well detected amyloid-β pathology and neuropathological AD change, supporting their use to detect amyloid-β pathology.

Although current AD research and clinical criteria consider amyloid-β PET or CSF interchangeable,[63–65] the AUC for amyloid-β PET[1] and CSF[66] are partially different. More specifically, the AUC for CSF includes all the above mentioned criteria for the PET AUC, but also includes cases with SCD, probable AD and behavioural change with AD as a suspected cause,[66] based on the assumption that CSF could be routinely used for a large proportion of cases in a dementia centre. However, as the PET AUC were created in 2013 to implement clinical amyloid-β PET, it is possible that more criteria could be added in the future.

In general, we didn't find specific reasons for current clinical practice to favour one modality over the other, especially as CSF $A\beta_{42}$ is often interpreted with tau proteins, increasing both its accordance to amyloid PET and specificity for clinical AD pathology. The choice between the modalities could depend on patient characteristics (possible co-pathologies such as NPH, anti-coagulant therapy, claustrophobia, fear for intervention), the preferences of the clinician and the patient, and cultural factors. In the future, if there would be an anti-amyloid disease modifying therapy most effective at the very earliest stages of the disease, our results suggest that CSF could be the modality of choice for patient selection. However, if future interventions require tracking amyloid-β load longitudinally, amyloid-β PET would be most appropriate.

METHODOLOGICAL CONSIDERATIONS AND LIMITATIONS

Several methodological issues need to be considered when interpreting results presented in this thesis.

Comparison of any two diagnostic tests

There is always variability in diagnostic tests, which makes investigating the possible different information provided by two different modalities complex. Therefore, even when comparing two highly correlated and widely used tests that are considered interchangeable in guidelines, there is expectedly some noise or variation between the two modalities. Although in this thesis we found evidence that group-wise discordant amyloid-β CSF/PET status, especially CSF+/PET-, are associated with clinically and biologically meaningful outcomes, a part of the CSF/PET discordance is certainly caused by variation. However, it is challenging to completely disentangle the possibly unique information of the discordant cases from methodological variation. As such, although some characteristics might be present at a group level, direct translation to the individual level may be complicated. Below, I will briefly discuss some factors that can influence the PET and CSF results, possibly causing one of the modalities to be false-positive or false-negative for amyloid-β pathology and thereby leading to amyloid-β CSF/PET discordance.

Methodological factors influencing CSF positivity

CSF biomarker values can be affected by a variety of factors. Most significantly, there has historically been a lack of standardization of CSF handling procedures between different CSF laboratories, which has made the development of global assay-specific cut-offs challenging.[67] Of these preanalytical factors, storage tube material has most evidence to have an effect on $A\beta_{42}$, although storage temperature, non-frozen storage time, and additives, such as detergents, blood contamination and centrifugation could also play a role.[68] To combat this, several initiatives have been formed to harmonize the procedures.[69,70]

The choice of analytical assay can influence the $A\beta_{42}$ levels[71,72] and there is also some intrinsic variability while using the same assay. In a study using ELISA and xMAP platforms (the same technologies were used also to obtain CSF $A\beta_{42}$ levels in our studies), the within-runs mean coefficients of variation (CV) were less than 4% for ELISA and 1.9%-7.4% for xMAP, as opposed to the much larger between laboratories CV in the 20-30% range.[73] Newer fully automated platforms have reported to have lower CV values leading to less variability.[74,75] If similar procedures are used with the

same assay over time, these factors should not have an effect on the longitudinal evaluation of $A\beta_{42}$ in the same cohort. However, in ADC there was a longitudinal upwards drift of median values of $A\beta_{42}$, possibly due to changes in ELISA kits and/or calibration data, illustrating also the methodological effects on within-cohort longitudinal data.[5,6] Finally, patient specific factors, such as intrinsic Aβ peptide production,[76] repeated lumbar punctures[77] or even sleep deprivation[78] might play a role.

CSF positivity: $A\beta_{42}$ vs ratios

We derived CSF amyloid-β status using CSF $A\beta_{42}$ levels with cohort-specific cut-offs. In the literature, alternative CSF measures have been used when comparing concordance to amyloid-β PET. More specifically, ratios of CSF $A\beta_{42}$ with either CSF (p)tau[72,79] or CSF $A\beta_{40}$[72,79,80] have been shown to increase concordance with amyloid-β PET status as compared to using solely CSF $A\beta_{42}$. Using CSF $A\beta_{40}$ levels allows to adjust for the intrinsic Aβ peptide levels, so that, for example, subjects with constitutively low Aβ peptide levels would not be falsely diagnosed with amyloid pathology.[76] Also, as $A\beta_{42}$ and $A\beta_{40}$ are both affected by pre-analytical factors, such as tube surface, using the ratio might alleviate this issue.[76] Using ratios with CSF tau adjusts for the existence of tau pathology, which by current definition, follows amyloid-β pathology.[81] If CSF $A\beta_{42}$ shows earlier amyloid-β pathology than PET, then adjusting for other biomarkers (such as CSF tau) later in the disease course would also improve concordance with PET.[82] In clinical practice, CSF $A\beta_{42}$ and tau proteins are often considered together, because AD is associated with changes in both protein levels. As the main goal of this thesis was to compare PET and CSF in capturing amyloid-β pathology, the main analysis was always performed without taking CSF tau into account. $A\beta_{40}$ levels were not available for our studies, reducing our capabilities to adjust for intrinsic Aβ peptide levels.

Methodological factors influencing PET positivity

Amyloid-β PET can be influenced by several factors. The most widely used approach for amyloid-β PET is standardized uptake value ratio (SUVR) images, derived from static acquisition.[83] This involves computation of the average signal from a late time frame normalized by the signal in a region not affected by amyloid-β pathology. The choice of reference region can be important. Cerebellar cortex, most often used for amyloid-β PET, is free of amyloid-β pathology at the earlier stages of sporadic AD, but gets involved at the latest AD stages[84] and in some forms of familial AD.[83] Subcortical white matter regions may result in more accurate longitudinal assessment,[85] however this white matter binding is poorly understood[86] and it might be susceptible to unspecific white matter lesions, associated with aging.

Alternatively, dynamic acquisition starting from the tracer injection can be used, allowing to create fully quantitative (e.g. non-displaceable binding potential [BP_{ND}]) images. Although overall these two methods are highly correlated,[87,88] it has also been shown that SUVR images can overestimate true binding and varies more in longitudinal studies,[89] while BP_{ND} may be more reliable for detecting early amyloid pathology in visual read.[90,91] Overall, amyloid-β PET has been shown to have <5% test-retest variability.[92,93]

Different tracers are used for amyloid-β PET. Newer tracers labelled with [^{18}F], which can also be used in centres without a cyclotron, tend to show greater unspecific binding in the white matter,[94,95] possibly causing false-positive cortical signal. In addition, tracer-specific guidelines differ for visual read, using different imaging planes and colour scales.[96] The choice of scanner and its performance can have a role. Additionally, PET signal can be affected by patient motion during the scan and significant brain atrophy causing shrinkage of areas with high uptake.[83] Finally, different centres have different image processing pipelines, which can introduce variability. Similarly to CSF, there have been initiatives to standardize PET procedures and interpretation[97] and to allow cross-tracer comparisons.[98]

PET positivity: visual read vs quantitative threshold

In most of our work, amyloid-β PET positivity was determined by visual read, which is the current clinical standard. For that, a scan is considered either positive of negative for amyloid-β based tracer uptake in the cortex, and also based on the striatum in case of [^{18}F]flutemetamol. In **chapter V**, however, we used a quantitative threshold based on a neocortical composite SUVR that was defined in a large autopsy study.[99] Although in the majority of cases they are concordant[100], there are two major differences between these methods. In visual read, amyloid positivity is determined based on just one positive region. This might theoretically evade detection by a larger composite region, which is the current standard for quantitative threshold approaches. Second, detection of high signal is based on the impression of the reader in visual read, whereas it is determined based on a cut-off for threshold methods. Therefore, quantitative threshold methods are highly dependent on using a correct cut-off, while visual read might also be affected by reader experience and underlying biases leading to increased inter- and intra-rater variability, especially when rating dubious / borderline scans. As processing steps are required for threshold measures, they are processing pipe-line dependent, more susceptible to errors, for example due to slight movement artifacts, atrophy and misregistration. Visual read has also been suggested to be slightly more conservative compared to threshold measures although this has recently been challenged.[19,101] Finally, the current standard for visual read provides a binary result, whereas

quantitative threshold methods provide a measurement on a continuous scale, including more information by definition.

It is possible that in the future smaller composite ROIs more specific for early amyloid-β pathology will be used to increase the sensitivity of quantitative threshold methods.[102] Also, visual read could be augmented with quantitative data to additionally include a continuous measure of amyloid-β load. Finally, artificial intelligence algorithms have been shown to have excellent accordance with visual read,[103] offering another possibility to determine amyloid-β PET status in the future.

Study populations

The majority of studies presented in this thesis were performed using data from the ADC, which is a large mixed memory clinic cohort in an academic setting.[104] On average, patients in the ADC are relatively young and at an earlier stage of the disease. Although CSF analysis is offered to all patients, amyloid-β PET was usually performed for research[52–56] and therefore its availability is dependent of inclusion criteria of different projects. Analyses presented in **chapter V** were performed using publicly accessible data from the multi-centre ADNI cohort, which includes longitudinal multimodal amyloid-β and tau biomarker data. Participants in ADNI were mainly recruited by advertisements; the participants were mostly Caucasian and well-educated.[56] These cohort characteristics might reduce the generalizability of our findings.

The nature of cut-offs and binarized status

In this thesis we focused on the concordance based on the binarized CSF/PET status, as opposed to the correlation between two continuous variables. While using binarized status is in line with clinical decision-making, and perhaps with also human thought patterns, it does not necessarily best describe the underlying process. A pathological value just over the cut-off might represent early pathology and might not be interchangeable with another value far over the cut-off associated with advanced pathology. In addition, there are different methods for determining cut-offs, each with their advantages and disadvatanges.[105] For example, the CSF $A\beta_{42}$ cut-off in the ADC was determined via Gaussian mixture modelling,[6] we additionally used Youden index for the tau/$A\beta_{42}$ ratio in **chapter II**, in **chapter V** we used cut-offs previously established differentiating AD from non-AD neuropathological diagnosis[99,106] and cut-offs based on the upper range of the reference group.[92,107] Using different cut-offs (or methods for deriving cut-offs) would result in different concordance.

Subthreshold pathology

While current cut-offs, for example determined based on differentiating advanced AD pathology from cognitively normal population might work well in clinical practice, different more sensitive cut-offs could be needed when aiming to capture beginning amyloid-β pathology for possible future anti amyloid-β therapies. To that vein, recently attention has been drawn to changes below the standard cut-offs. For example, amyloid-β pathology has been linked with subtle memory changes already in the subthreshold PET range.[108,109] Therefore is it possible, that in some CSF+/PET- patients there is already some increased PET signal, which is undetectable by current ROI techniques and cut-offs although data using visual assessment in this group were not available. Using different more sensitive cut-offs to capture this early change, however, could result in reduced specificity.

One possible solution is to utilize a more stringent cut-off to detect certain pathology, and a lenient cut-off to detect early changes, and to consider the area between these two cut-offs as an unspecific grey zone.[110] Compared to binarization, this would likely give a better estimation of the biological complexity. However, this would also result in quite a large peri-threshold group, of whom it is unclear, whether they have real underlying (amyloid-β) pathology or not. It has been shown that on a group level grey zone amyloid-β PET burden has subtle effects on cognitive decline, suggesting that this approach partly captures pathology below the conventional thresholds.[111] For the individual, however, additional diagnostic tests would be needed to certify the underlying pathology, demanding more resources. Even if binarized status is not a perfect model, it might be a reasonable simplification, especially in a clinical setting.

Although the grey zone approach is most often discussed regarding quantitative amyloid-β PET, it is also applicable to PET visual read, CSF $A\beta_{42}$ and other biomarkers. An estimation of how amyloid-β CSF/PET discordance is intertwined with the gray zone approach for both modalities is presented in **Figure 1**. This model captures the main themes presented in this thesis, such as CSF+/PET- being early biomarker for amyloid-β pathology, methodological variation and the distance from the cut-off being helpful for the diagnostic certainty of the amyloid-β status. Note that the final 'grey zone' when using both amyloid-β modalities is smaller than it would be for either of the two modalities separately. The CSF+ group with perithreshold PET signal is likely the most robust early amyloid-β group. It is unclear what is the amyloid-β status of the 'true CSF-/PET+' group, but in our experience these cases are exceedingly rare. As using grey zones when comparing biomarkers would result in at least nine subgroups, validation of this model would require large sample sizes.

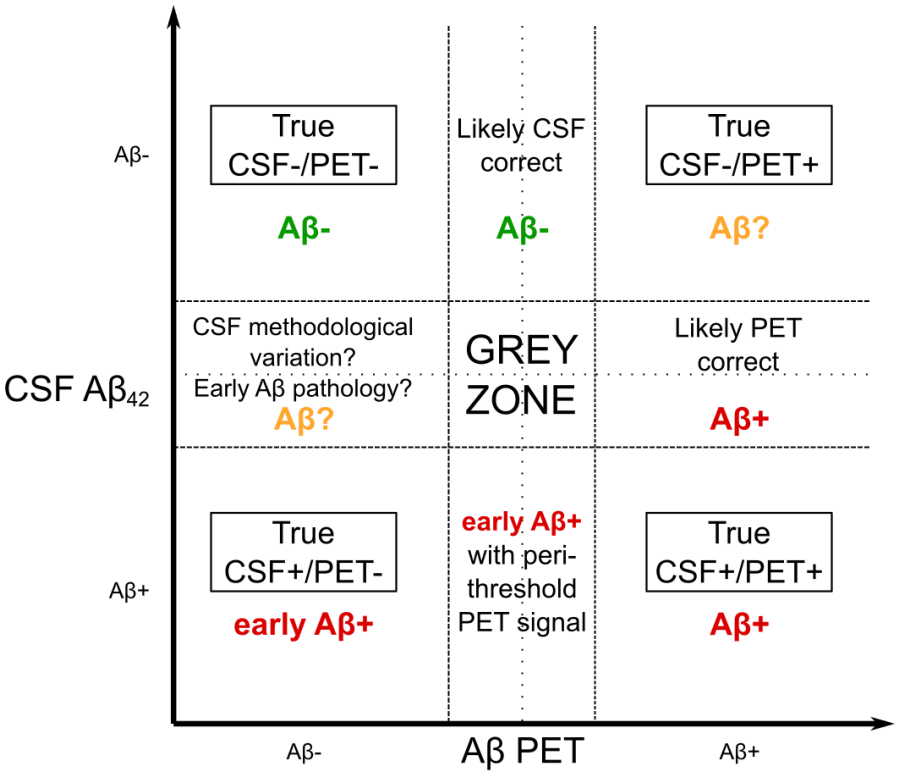

Figure 1. Grey zone approach for amyloid-β (Aβ) CSF/PET discordance

FUTURE PERSPECTIVES

It is difficult to completely disentangle the possibly unique information of the CSF/PET discordant cases from methodological variation. Future improvements for both CSF and PET methodologies including new criteria for visual assessment could decrease variability and increase patient-level certainty. As suggested, the distance from the cut-off of amyloid-β positivity is one indicator that might give additional information in these cases. Perhaps in the future other early biomarkers are found that could, in combination with amyloid-β status, increase patient-level confidence. Longer follow-up times in future studies would also be helpful to determine the proportion of CSF+/PET- cases who convert to CSF+/PET+ and the clinical consequences. Although not yet shown, it is probable that over time the cognitive trajectories of CSF+/PET- diverge from the CSF-/PET- group. Amyloid-β CSF+/PET- subjects could provide an interesting target population for clinical trials, although undergoing two amyloid-β biomarkers might not be feasible.

Neuropathology can provide an opportunity to investigate CSF/PET discordance, although it is rare to have cases with CSF, amyloid-β PET and autopsy available. Our findings from **chapter VI** should be validated in other cohorts and to find whether there are more neuropathological substrates to the discordance, such as neuropathological distribution, morphology and load of amyloid-β. For example, it has been shown that there might be structural variation in amyloid-β fibrils in different AD subtypes,[112] and structural variation could possibly influence CSF/PET discordance.[113] Other brain amyloidoses could provide an additional avenue to investigate amyloid-β CSF and PET biomarkers. Finally, there have been previous work investigating the possible discordant CSF/PET information for tau pathology.[11,114] Further research on that subject could also give more insight into the possible different information gained by amyloid-β CSF/PET.

Recent advances have been made in developing plasma amyloid-β diagnostics.[115–117] Future studies exploring the concordance between plasma, CSF and PET would be useful to detect whether different discordant patterns include additional information, and whether plasma and CSF amyloid-β positivity develop at the same time.

CONCLUSION

In this work we took different approaches to investigate the potentially different information provided by amyloid-β PET and CSF and the consequences of having discordant CSF/PET status. We used different cohorts and modelling techniques, investigated patient charts, utilized longitudinal biomarker results and neuropathological data. In biology, as well as everywhere in life, there are no easy answers to complex questions. Similarly, amyloid-β CSF/PET discordancy is likely a result of various factors, both methodological and biological. Of these different possible explanations, longitudinal accumulation of amyloid-β in CSF+/PET- without significant tau pathology nor cognitive decline indicates that amyloid-β CSF+/PET- is a marker for early amyloid-β pathology. The most important current clinical implication of our work is that amyloid-β CSF/PET discordant patients seem to have more benign prognosis compared to those with concordant positive amyloid-β status. When (and not *if*) an effective anti-amyloid disease modifying therapy has become available, our results might also be relevant for patient selection.

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APPENDIX

ABBREVIATIONS

Aβ	Amyloid-β
AD	Alzheimer's disease
ADC	Amsterdam Dementia Cohort
ADNI	Alzheimer's Disease Neuroimaging Initiative
AIC	Akaike information criterion
APOE	Apolipoprotein E
APP	Amyloid precursor protein
AUC	Area-under-the-curve
AUC	Appropriate-use-criteria
CAA	Cerebral amyloid angiopathy
CL	Centiloid
CSF	Cerebrospinal fluid
CSF1R	Colony stimulating factor 1 receptor
CT	Computed tomography
CV	Coefficients of variation
FDG	[^{18}F]Fluorodeoxyglucose
FDR	False discovery rate
FTLD	Frontotemporal lobar degeneration
GCA	Global cortical atrophy
HLDS	Adult-onset leukoencephalopathy with axonal spheroids
IQR	Interquartile range
LP	Lumbar puncture
MCI	Mild cognitive impairment
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging
MTA	Medial temporal atrophy
NPH	Normal-pressure hydrocephalus

p-tau	Phosphorylated tau
PCA	Posterior cortical atrophy
PET	Positron emission tomography
PIB	[^{11}C]Pittsburgh Compound B
ROI	Region of interest
SCD	Subjective cognitive decline
SD	Standard deviation
SUVR	Standardized uptake value ratio
t-tau	Total tau
TMT	Trail Making Test
UNC	Uncorrected
VAT	Visual Association Test
VIM	Variable importance measure
WHO	World Health Organization

ABSTRACT (ENG)

Discordance between amyloid-β PET and CSF biomarkers: Clinical and pathophysiological consequences

Among the earliest neuropathological events in Alzheimer's disease (AD) is the accumulation and aggregation of amyloid-β, which occurs decades before symptom onset. Two methods, amyloid-β positron emission tomography (PET) and $A\beta_{42}$ levels in the cerebrospinal fluid (CSF), are currently considered interchangeable for assessing amyloid-β pathology *in vivo*. However, in 10-20% of cases they show discordant (CSF+/PET- or CSF-/PET+) results. The causes for this discordance are largely unknown.

In this thesis we used several different approaches, both in vivo and ex vivo, to investigate the possible independent information provided by amyloid-β PET and CSF. First, we investigated the clinical consequences of having discordant amyloid-β CSF/PET biomarker status. Then we focused on the potential differences of the CSF+/PET- and CSF-/PET+ participants by utilizing the predictive ability of various patient features. Next, we explored why sometimes amyloid-β PET is clinically requested for patients with available CSF biomarker analysis. Thereafter, we investigated longitudinal trajectories of amyloid-β accumulation, tau and cognition and studied whether amyloid-β CSF/PET discordant status is associated with tau 5 years later. Finally, we investigated the neuropathological underpinnings of amyloid-β CSF/PET concordance in a sample with available neuropathological data.

We found that there was consistently ~15% discordance between amyloid-β PET and CSF in different samples and cohorts. The discordance rate differed by disease stage, being more frequent in early disease. Amyloid-β CSF+/PET- status was consistently more prevalent than CSF-/PET+. Although discordant amyloid-β status is not benign and is associated with amyloid-β pathology, it is associated with a distinctly better prognosis compared to having concordant positive amyloid-β biomarkers before dementia.

Our results combined with previous work from others suggest that amyloid-β CSF/PET discordance is likely caused by different factors, both biological and methodological. We found that on a group level CSF+/PET- status is associated with early amyloid-β pathology where significant neuronal loss has not yet begun, offering opportunities for future trials. CSF+/PET- status could also be caused by other diseases, such as neuroinflammation, and methodological variation. Cases with CSF-/PET+ status are most often caused by a combination false-negative CSF in cases of AD, and false-positive PET signal in cases without AD. Although CSF and PET might not offer identical information about amyloid-β pathology, in the large majority of cases they are

concordant and both can be used to assess *in vivo* amyloid-β pathology. In complicated clinical dilemmas, there even might be complementary value of using both biomarkers.

In conclusion, our results offer insights to the pathophysiology of Alzheimer's disease and the clinical utility of *in vivo* amyloid-β biomarkers.

LÜHIKOKKUVÕTE (EE)

Amüloid-β staatuse vastuolu PET-uuringul ning liikvorianalüüsil: kliiniline ja patofüsioloogiline tähendus

Amüloid-β ladestumine peaajus on üks varasemaid neuropatoloogilisi muutusi Alzheimeri tõve (AD) puhul. Kaasaegsete teadmiste kohaselt toimub amüloid-β ladestumine juba aastakümneid enne haiguse sümptomite avaldumist. Amüloid-β tuvastamiseks peaajus *in vivo* on tänapäeval enim kasutatud kaks uuringumeetodit: positronemissioon-tomograafia (PET) ning $A\beta_{42}$ taseme määramine liikvorianalüüsis (*cerebrospinal fluid*, CSF). Neid kahte diagnostilist meetodit peetakse tavapäraselt võrdväärseteks alternatiivideks, kuid uuringutes on korduvalt näidatud, et 10-20% juhtudest annavad nad vastuolulise (CSF+/PET- või CSF-/PET+) tulemuse, mille põhjuste kohta ei ole kogutud piisavalt andmeid.

Käesolevas väitekirjas kasutasime nii *in vivo* kui *ex vivo* meetodeid, et uurida, millisel määral ja millistel patsientidel nende kahe diagnostilise meetodi kasutamisel saadud informatsioon erineb. Esimesena uurisime, kas vastuolulisel CSF/PET amüloidstaatusel on kliiniline tähendus. Seejärel, kasutades erinevate patsienditunnuste prognoosivõimet, võrdlesime kaht vastuolulist amüloid-β gruppi (CSF+/PET- ning CSF-/PET+). Siis uurisime, miks kliinilises praktikas tellitakse siiski amüloid-β PET-uuring, kui liikvorianalüüs on juba sooritatud. Järgmisena jälgisime, kuidas amüloid-β CSF/PET vastuolulise staatusega patsientidel kogunevad ajus nii amüloid-β kui tau-valk ning kuidas muutub selliste patsientide kognitiivne võimekus. Lõpetuseks kasutasime lahanguandmeid, et uurida, millised neuropatoloogilised muutused põhjustavad vastuolulist amüloid-β CSF/PET staatust.

Uuringute tulemusena leidsime, et eri uuringugruppides olid PET ja CSF tulemused järjepidevalt ~15% juhtudest vastuolulise tulemusega. Vastuolulisi CSF/PET tulemusi esines rohkem varasemates AD staadiumites. CSF+/PET- staatust täheldasime järjepidevalt rohkem kui CSF-/PET+ staatust. Kuigi vastuoluline amüloid-β CSF/PET staatus on seotud amüloidpatoloogiaga, on vastuolulise CSF/PET tulemusega mittedementsete patsientide prognoos oluliselt parem, võrreldes nendega, kellel mõlemad uuringud näitavad amüloidpatoloogia olemasolu.

Meie tulemused osutavad, et amüloid-β CSF/PET vastuolulised tulemused on põhjustatud nii bioloogiliste kui metoodiliste tegurite poolt. Me leidsime, et grupitasandil näitab CSF+/PET- staatus varajast amüloid-β patoloogiat. See tulemus võib osutuda oluliseks tulevikus ravimuuringute puhul, kui tahetakse uuringusse haarata väga varajases staadiumis AD patsiente. Vastuolulist CSF+/PET- staatust võivad põhjustada mõned muud haigused (näiteks autoimmuunsed põletikud) ning metoodikast tulenev variatsioon. Amüloid-β CSF-/PET+ staatust võivad põhjustada valenegatiivne liikvorianalüüs AD puhul ning valepositiivne PET-uuring

amüloidpatoloogiata inimestel. Kuigi liikvorianalüüs ning PET-uuring ei anna amüloidpatoloogia kohta täielikult kattuvat informatiooni, on nad valdavas enamuses haigusjuhtudest siiski omavahel kooskõlas ning mõlemat meetodit saab kasutada amüloid-β diagnostikas. Meie tulemused näitavad, et keerulisematel kliinilistel haigusjuhtudel võib olla näidustatud ka mõlema uuringu teostamine.

Kokkuvõtteks, meie tulemused pakuvad teadmisi nii Alzheimeri tõve patofüsiloogia kui *in vivo* amüloid-β biomarkerite kliinilise kasutuse kohta.

SAMENVATTING (NL)

Discordantie tussen amyloïde-β PET- en CSF-biomarkers: klinische en pathofysiologische gevolgen

Een van de eerste neuropathologische veranderingen bij de ziekte van Alzheimer (ZvA) is de accumulatie en aggregatie van het amyloïd-β eiwit, wat tientallen jaren vóór het begin van de symptomen optreedt. De twee methoden voor het *in vivo* beoordelen van amyloïd-β-pathologie, namelijk amyloïd-β positron emissie tomografie (PET) en de concentratie $A\beta_{42}$ in het hersenvocht (cerebrospinale vloeistof [CSF]), worden momenteel als onderling verwisselbaar beschouwd. Echter, in 10-20% van de gevallen zijn de resultaten tegenstrijdig (CSF+/PET- of CSF-/PET+). De oorzaken hiervan zijn grotendeels onbekend.

In dit proefschrift hebben we verschillende benaderingen gebruikt, zowel *in vivo* als *ex vivo*, om de mogelijk onafhankelijke informatie van amyloïd-β PET en CSF te onderzoeken. Eerst onderzochten we de klinische gevolgen van een discordante amyloïde-β CSF/PET biomarker status. Vervolgens hebben we ons gericht op de mogelijke verschillen tussen CSF+/PET- en CSF-/PET+ deelnemers door gebruik te maken van het voorspellende vermogen van verschillende patiëntkenmerken. In een klinische setting, hebben we onderzocht waarom voor patiënten met een al beschikbare CSF-biomarker, een amyloïd-β PET soms alsnog werd aangevraagd. Daarna onderzochten we zowel de longitudinale ontwikkeling van amyloïd-β accumulatie, tau en cognitief vermogen en de associatie tussen amyloïde-β CSF/PET discordantie met tau pathologie 5 jaar later. Ten slotte, onderzochten we de neuropathologische onderbouwing van amyloïd-β CSF/PET concordantie in een *post-mortem* dataset.

We observeerden dat er consistent ~15% discordantie was tussen amyloïde-β PET en CSF in verschillende datasets en cohorten. De mate van discordantie verschilde per ziektestadium en kwam vaker voor in de vroege fase van de ziekte. Over het algemeen, kwam amyloïde-β CSF+/PET-status frequenter voor dan CSF-/PET+ status. Hoewel een discordante amyloïde-β status niet goedaardig is en wordt geassocieerd met amyloïde-β pathologie, is de prognose duidelijk beter dan bij een concordante positieve status.

Onze resultaten, gecombineerd met eerder werk van anderen, suggereren dat amyloïde-β CSF/PET discordantie waarschijnlijk wordt veroorzaakt door verschillende factoren, zowel biologisch als methodologisch. We zagen dat CSF+/PET- status op groepsniveau geassocieerd is met vroege amyloïde-β pathologie, waarbij mogelijk significant neuronaal verlies nog niet begonnen is. Dit biedt kansen voor toekomstige studies. De CSF+/PET- status kan ook worden veroorzaakt door andere ziekten, zoals neuro-inflammatie en methodologische variatie. Daarentegen, gevallen met

CSF-/PET+ status worden meestal veroorzaakt door een combinatie van vals-negatieve CSF en vals-positief PET-signaal in patiënten respectievelijk met en zonder ZvA. Hoewel CSF en PET mogelijk geen identieke informatie bieden over amyloïde-β pathologie, zijn ze in de meeste gevallen concordant en kunnen beide worden gebruikt om *in vivo* amyloïde-β pathologie te beoordelen. Bij gecompliceerde klinische dilemma's kan het zelfs complementair zijn om beide biomarkers te gebruiken.

Concluderend, bieden onze resultaten inzicht in de pathofysiologie van de ziekte van Alzheimer en de klinische bruikbaarheid van *in vivo* amyloïde-β biomarkers.

LIST OF PUBLICATIONS

In the thesis

de Wilde A*, **Reimand J***, Teunissen CE, Zwan M, Windhorst AD, Boellaard R, van der Flier WM, Scheltens P, van Berckel BNM, Bouwman F, Ossenkoppele R. Discordant amyloid-β PET and CSF biomarkers and its clinical consequences. Alzheimers Res Ther. 2019 Sep 12;11(1):78.

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Reimand J, Groot C, Teunissen CE, Windhorst AD, Boellaard R, Barkhof F, Nazarenko S, van der Flier WM, van Berckel BNM, Scheltens P, Ossenkoppele R, Bouwman F. Why Is Amyloid-β PET Requested After Performing CSF Biomarkers? J Alzheimers Dis. 2020;73(2):559-569.

Reimand J, Collij L, Scheltens P, Bouwman F, Ossenkoppele Rik. Association of amyloid-β CSF/PET discordance and tau load five years. Neurology. September 2020:10.1212/WNL.0000000000010739

Reimand J, Boon BCD, Collij LE, Teunissen CE, Rozemuller AJM, van Berckel BNM, Scheltens P, Ossenkoppele R, Bouwman F. Amyloid-β PET and CSF in an autopsy confirmed cohort. Ann Clin Transl Neurol. October 2020:acn3.51195. doi:10.1002/acn3.51195

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Other publications

Collij LE, Konijnenberg E, **Reimand J**, Kate MT, Braber AD, Lopes Alves I, Zwan M, Yaqub M, van Assema DME, Wink AM, Lammertsma AA, Scheltens P, Visser PJ, Barkhof F, van Berckel BNM. Assessing Amyloid Pathology in Cognitively Normal Subjects Using 18F-Flutemetamol PET: Comparing Visual Reads and Quantitative Methods. J Nucl Med. 2019 Apr;60(4):541-547.

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Visser D, Wolters EE, Verfaillie SCJ, Coomans EM, Timmers T, Tuncel H, **Reimand J**, Boellaard R, Windhorst AD, Scheltens P, van der Flier WM, Ossenkoppele R, van Berckel BNM. Tau pathology and relative cerebral blood flow are independently associated with cognition in Alzheimer's disease. Eur J Nucl Med Mol Imaging. May 2020.

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how_to_apply/ADNI_Acknowledgement_List.pdf

PORTFOLIO

Some listed activities are represented in the portfolios of both universities.

Alzheimercentrum, VUmc

	Year	ECTs
Courses		
VU-NT2 Dutch for work and study (levels 2.2, 3, 4)	2018 - 2019	9.0
Managerial Psychology	2018	4.0
Scientific Integrity	2018	2.0
BROK course	2018	1.5
BETA ONWAR – Neurodegeneration of the Nervous System	2018	1.4
Statistics with R	2018	6.0
Conferences		
Alzheimer Association International Conference (oral)	2020	2.0
Neuroscience Campus Amsterdam annual meeting	2019	0.29
Dementia Update	2019	0.3
Human Amyloid Imaging (poster)	2019	2.0
European Course in Neuroradiology – Trauma and Degenerative Diseases	2018	1.45
Alzheimer Association International Conference (oral)	2018	2.0
Neuroscience Campus Amsterdam annual meeting	2018	0.3
Dementie Update	2018	0.3
Other		
Clinical work	2018 - 2020	10.0
Participation in the Friday afternoon program	2018 - 2020	4.0

Department of Health Technologies, TalTech

	Year	ECTs
Module: General Studies		
Modern Medical Technology Management Strategies (Continuous training)	2019	2.0
Theory and Ethics in Medicine (Continuous training)	2019	3.0
Managerial Psychology	2018	4.0
Module: Basic Studies		
Clinical Laboratory Methods (Continuous training)	2018	2.0
Mathematics for Doctoral Students I (Continuous training)	2018	5.0
Module: Special studies 1bm		
Doctoral Workshop on Biomedical Engineering	2019	6.0
Professional Training	2018	6.0
Module: Special studies 2bm		
Presentations on Specialty	2019	4.0
Preparation of Scientific Reports	2019	6.0
Doctoral Seminar on Bioelectromagnetism	2018	6.0

LIST OF DISSERTATIONS – ALZHEIMERCENTRUM, VUMC

1. L. Gootjes: Dichotic Listening, hemispheral connectivity and dementia (2004)
2. K. van Dijk: Peripheral Nerve Stimulation in Alzheimer's Disease (2005)
3. R. Goekoop: Functional MRI of cholinergic transmission (2006)
4. R. Lazeron: Cognitive aspects in Multiple Sclerosis (2006)
5. N.S.M. Schoonenboom: CSF markers in Dementia (2006)
6. E.S.C. Korf: Medial Temporal Lobe atrophy on MRI: risk factors and predictive value (2006)
7. B. van Harten: Aspects of subcortical vascular ischemic disease (2006)
8. B. Jones: Cingular cortex networks: role in learning and memory and Alzheimer's disease related changes (2007)
9. L. van de Pol: Hippocampal atrophy from aging to dementia: a clinical and radiological perspective (2007)
10. Y.A.L. Pijnenburg: Frontotemporal dementia: towards an earlier diagnosis (2007)
11. A.Bastos Leite:Pathological ageing of the Brain (2007)
12. E.C.W. van Straaten: Vascular dementia (2008)
13. R.L.C. Vogels: Cognitive impairment in heart failure (2008)
14. J. Damoiseaux: The brain at rest (2008)
15. G.B. Karas: computational neuro-anatomy (2008)
16. F.H. Bouwman: Biomarkers in dementia: longitudinal aspects (2008)
17. A.A. Gouw: Cerebral small vessel disease on MRI: clinical impact and underlying pathology (2009)
18. H. van der Roest: Care needs in dementia and interactive digital information provisioning (2009)
19. C. Mulder: CSF Biomarkers in Alzheimer's disease (2009)
20. W. Henneman. Advances in hippocampal atrophy measurement in dementia: beyond diagnostics (2009)
21. S.S. Staekenborg: From normal aging to dementia: risk factors and clinical findings in relation to vascular changes on brain MRI (2009)
22. N. Tolboom: Imaging Alzheimer's disease pathology in vivo: towards an early diagnosis (2010)
23. E. Altena: Mapping insomnia: brain structure, function and sleep intervention (2010)
24. N.A. Verwey: Biochemical markers in dementia: from mice to men. A translational approach (2010)
25. M.I. Kester: Biomarkers for Alzheimer's pathology; Monitoring, predicting and understanding the disease (2011)
26. J.D. Sluimer: Longitudinal changes in the brain (2011)
27. S.D Mulder: Amyloid associated proteins in Alzheimer's Disease (2011)
28. S.A.M. Sikkes: measuring IADL in dementia (2011)
29. A Schuitemaker: Inflammation in Alzheimer's Disease: in vivo quantification (2012)

30. K. Joling: Depression and anxiety in family caregivers of persons with dementia (2012)
31. W. de Haan: In a network state of mind (2012) (Cum Laude)
32. D. van Assema: Blood-brain barrier P-glycoprotein function in ageing and Alzheimer's disease (2012)
33. J.D.C. Goos: Cerebral microbleeds: connecting the dots (2013)
34. R. Ossenkoppele: Alzheimer PEThology (2013)
35. H.M. Jochemsen: Brain under pressure: influences of blood pressure and angiotensin- converting enzyme on the brain (2013)
36. A.E.van der Vlies: Cognitive profiles in Alzheimer's disease: Recognizing its many faces (2013)
37. I. van Rossum: Diagnosis and prognosis of Alzheimer's disease in subjects with mild cognitive impairment (2013)
38. E.I.S. Møst: Circadian rhythm deterioration in early Alzheimer's disease and the preventative effect of light (2013)
39. M.A.A. Binnewijzend: Functional and perfusion MRI in dementia (2014)
40. H. de Waal: Understanding heterogeneity in Alzheimer's disease: A neurophysiological perspective (2014)
41. W. Jongbloed: Neurodegeneration: Biochemical signals from the brain (2014)
42. E.L.G.E. Poortvliet-Koedam: Early-onset dementia: Unraveling the clinical phenotypes (2014)
43. A.C. van Harten: The road less traveled: CSF biomarkers for Alzheimer's disease:
44. Predicting earliest cognitive decline and exploring microRNA as a novel biomarker source (2014)
45. A.M. Hooghiemstra: Early-onset dementia: With exercise in mind (2014)
46. L.L. Sandberg-Smits: A cognitive perspective on clinical manifestations of Alzheimer's disease (2015)
47. F.H. Duits: Biomarkers for Alzheimer's disease, current practice and new perspectives (2015)
48. S.M. Adriaanse: Integrating functional and molecular imaging in Alzheimer's disease (2015)
49. C. Möller: Imaging patterns of tissue destruction – Towards a better discrimination of types of dementia (2015)
50. M. del Campo Milán: Novel biochemical signatures of early stages of Alzheimer's disease (2015)
51. M. R. Benedictus: A vascular view on cognitive decline and dementia: relevance of cerebrovascular MRI markers in a memory clinic (2016)
52. M. D. Zwan: Visualizing Alzheimer's disease pathology. Implementation of amyloid PET in clinical practice (2016)
53. E. Louwersheimer: Alzheimer's disease: from phenotype to genotype (2016)
54. W.A. Krudop: The frontal lobe syndrome: a neuropsychiatric challenge (2016)

55. E.G.B. Vijverberg: The neuropsychiatry of behavioral variant frontotemporal dementia and primary psychiatric disorders: similarities and dissimilarities (2017)
56. F.T. Gossink: Late Onset Behavioral Changes differentiating between bvFTD and psychiatric disorders in clinical practice (2018)
57. M.A. Engels: Neurophysiology of Dementia (2018)
58. S.C.J. Verfaillie: Neuroimaging in subjective cognitive decline: Incipient Alzheimer's disease unmasked (2018)
59. M. ten Kate: Neuroimaging in Predementia Alzheimer's Disease (2018)
60. H.F.M. Rhodius-Meester: Optimizing use of diagnostic tests in memory clinics; the next step (24-09-2018)
61. E.A.J. Willemse: Optimizing biomarkers in cerebrospinal fluid. How Laboratory reproducibility improves the diagnosis of Alzheimer's disease (2018)
62. E. Konijnenberg : Early amyloid pathology – Identical twins, two of a kind ? (2019)
63. A.E. Leeuwis: Connecting heart and brain; Vascular determinants of cognitive impairment and depressive symptoms (2019)
64. J. Den Haan: Imaging The Retina in Alzheimer's Disease (2019)
65. A.C. van Loenhoud: Cognitive reserve in Alzheimer's disease. A perspective on the flourishing and withering of the brain (2019)
66. R.J. Jutten: Capturing changes in cognition; Refining the measurement of clinical progression in Alzheimer's disease (2019)
67. N. Legdeur: Determinants of cognitive impairment in the oldest-old (2019)
68. R. Slot: Subjective cognitive decline-predictive value of biomarkers in the context of preclinical Alzheimer's disease (2019)
69. N. Scheltens: Understanding heterogeneity in Alzheimer's disease-a data driven approach (2019)
70. L. Vermunt: Secondary Prevention for Alzheimer Disease – Timing, Selection and Endpoint of Clinical Trials (2020)
71. L.M.P. Wesselman: Lifestyle and brain health – exploring possibilities of an online intervention in non-demented elderly (2020)
72. I.S. van Maurik: Interpreting biomarker results in patients with mild cognitive impairment to estimate prognosis and optimize decision making (2020) (Cum Laude)
73. E. Dicks: Grey matter covariance networks in Alzheimer's disease: Edging towards a better understanding of disease progression (2020)
74. J.J. van der Zande: A sharper image of dementia with Lewy bodies: the role of imaging and neurophysiology in DLB, and the influence of concomitant Alzheimer's disease pathology (2020)
75. I. van Steenoven: Cerebrospinal fluid biomarkers in dementia with Lewy bodies – towards a biological diagnosis (2020)
76. N. Beker: Cognition in Centenarians – evaluation of cognitive health and underlying factors in centenarians from the 100-plus Study (2020)

77. F. de Leeuw: Nutrition and metabolic profiles in Alzheimer's disease (03-12-2020)
78. T. Timmers: Tau PET across the Alzheimer's disease continuum (02-12-2020)
79. E.E. Wolters: Untangling tau pathology using PET (02-12-2020)

LIST OF DISSERTATIONS – DEPARTMENT OF HEALTH TECHNOLOGIES, TALTECH

1. K. Meigas: Coherent Photodetection with a Laser (1997)
2. J. Riipulk: Microwave Radiometry for Medical Applications (2000)
3. T. Lipping: Processing EEG during Anaesthesia and Cardiac Surgery with Non-linear Order Statistics based Methods (2001)
4. J. Lass: Biosignal interpretation: Study of Cardiac Arrhythmias and Electromagnetic Field Effects on Human Nervous System (2002)
5. I. Fridolin: Photon Propagation in Tissue and in Biological Fluids (2003)
6. P. Lallo: Adaptive Secure Data Transmission Method for OSI Level 1 (2005)
7. F. Uhlin: Haemodialysis Treatment Monitored on-line by Ultra Violet Absorbance (2006)
8. R. Ferenets: EEG Patterns and Regularity Properties during Propofol Induced Anesthesia/Sedation (2007)
9. M. Bachmann: Effect of modulated microwave radiation on human resting electroencephalographic signal (2008)
10. M. Luman: Dialysis Dose and Nutrition Assessment by an Optical Method (2010)
11. P. Ross: Data Sharing and Shared Workflow in Medical Imaging (2011)
12. M. Tiik: Access Rights and Organizational Management in Implementation of Estonian Electronic Health Record System (2012)
13. A. Suhhova: Detection of the Effect of Weak Stressors on Human Resting Electroencephalographic Signal (2013)
14. J. Holmar: Optical Method for Uric Acid Removal Assessment During Dialysis (2013)
15. K. Pilt: Optical Pulse Wave Signal Analysis for Determination of Early Arterial Ageing in Diabetic Patients (2014)
16. A. Anier: Estimation of the Complexity of the Electroencephalogram for Brain Monitoring in Intensive Care (2014)
17. J. Arund: Major Chromophores and Fluorophores in the Spent Dialysate as Cornerstones for Optical Monitoring of Kidney Replacement Therapy (2016)
18. A. Šamarin: Hybrid PET/MR Imaging of Bone Metabolism and Morphology (2016)
19. V. Pille: Development of a Model for the Prevention of Work-Related Musculoskeletal Disorders in the Upper Extremities (2016)

20. R. Tomson: Urea- and Creatinine-Based Parameters in the Optical Monitoring of Dialysis: The Case of Lean Body Mass and Urea Rebound Assessment (2017)
21. M. Viigimäe: Analysis of Ventricular Repolarization Signals in Obstructive Sleep Apnea (2018)
22. S. Kalle: Optical Monitoring of Uremic Metabolites-Fluorophores during Dialysis: the Cases of β2-microglobulin, Pentosidine, and 4-Pyridoxic Acid (2018)
23. I. Mohammad: Development of Room Temperature Secondary Fluoride Ion Batteries: (2019)
24. E. Sõõru: Mismatch of sleep and work arrangements among research and development employees and personalisation of sleep studies (2019)
25. A. Talvik: Noninvasive Hemodynamic Monitoring as a Guide to Drug Treatment of Uncontrolled Hypertensive Patients: (2020)
26. P. Molaiyan: Solid-State Electrolytes for Fluoride-ion Batteries (2020)
27. K. Lauri: Elimination of Uremic Toxins during Dialysis assessed with the Optical and Analytical Methods (2020)
28. J. Teras: Loco-Regional Treatment of Cutaneous Melanoma (2020)

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CURRICULUM VITAE

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EDUCATION

2018 – 2021	PhD student (jointly between Alzheimercentrum, Vrije Universiteit Amsterdam, the Netherlands & Department of Health Technologies, Tallinn University of Technology, Estonia)
2016 – 2018	European Diploma of Neuroradiology (Pierre Lasjaunias Courses by the European Society of Neuroradiology)
2012 – 2017	Medical Residency in Radiology and Nuclear Medicine (University of Tartu, Tartu, Estonia)
2006 – 2012	Doctor of Medicine, MD (University of Tartu, Tartu, Estonia)

PROFESSIONAL EXPERIENCE

2017 –	Radiologist, subspecialisation in neuroimaging (Radiology Centre, North Estonia Medical Centre, Estonia)
2016 –	Chief Site Investigator of the “Enhancing Capacity of Neuroimaging and Biomarkers: Application in Early-stage Alzheimer's Disease with Comorbidities“ (coordinated by the International Atomic Energy Agency)

LANGUAGES

Estonian	Native language
English	Full professional proficiency
Dutch	Limited working proficiency
Russian	Elementary proficiency